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Specialized Localization of P-Glycoprotein Recognized by MRK 16 Monoclonal Antibody in Endothelial Cells of the Brain and the Spinal Cord

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This communication describes the cellular and ultrastructural localization in the central nervous system of P-glycoprotein (P-GP) recognized by a murine monoclonal antibody, MRK 16. At the ultrastructural level P-GP was strictly confined to the luminal surface of the endothelial cells which comprise the capillary vessels of the brain and the spinal cord. No P-GP was found in the endothelial cells of other organs. Our findings may be useful as a means to define the blood-brain barrier, and they imply that the blood-brain barrier is anatomically characterized by the presence of intercellular tight junctions between continuous nonfenestrated endothelial cells.

Key word: P-glycoprotein — MRK 16 — Endothelial cell — Brain — Spinal cord

P-glycoprotein (P-GP) is believed to transport various drugs out of the membranes of multidrug-resistant cells.[1,2] While we were investigating the systemic distribution of P-GP with an immunoperoxidase staining technique, we found that P-GP was expressed in cancerous tissues as well as normal tissues.[3,4] Furthermore, we noticed that some endothelial cells in the cerebrum were immunostained with MRK 16 MAb. This has already been reported by Cordon-Cardo *et al.*[5] and Thiebaut *et al.*[6] These findings prompted us to explore further the precise localization of P-GP in the central nervous system at the ultrastructural level.

Human non-cancerous tissues were obtained for diagnostic procedures from biopsies performed on patients at Saitama Medical Center, Saitama Medical School and Department of Pathology, Faculty of Medicine, the University of Tokyo, and from autopsies performed within 2 h of death on patients with various types of heart disease or other malignant conditions. Tissues for frozen section studies were immediately snap-frozen in liquid nitrogen and stored at −70°C until sectioning.

Frozen sections were prepared for immunoperoxidase staining (ABC-PO method) as described previously.[7] For these experiments, 100 μl of MRK 16 monoclonal antibody (MAb) (10 μg/ml) and nonimmune mouse serum (10 μg/ml) as a negative control were used. The characterization of MRK 16 MAb has already been described in detail.[8] To examine the localization in the brain of the P-GP recognized by MRK 16 MAb, an immunoelectron microscopical study was carried out.[3,7] An immunoprecipitation technique was used for the brain and the adrenal tissues to demonstrate the site of P-GP.[4,9]

We have already reported that P-GP can be recognized in placenta, proximal renal tubules, and adrenal cortices.[3,4] In addition, we have now found P-GP in the endothelial cells of the brain and the spinal cord (Table I and Fig. 1). Interestingly, the P-GP expression was limited to the capillary blood vessels, and was not present in relatively large blood vessels. No P-GP was recognized in the endothelial cells of other organs. We further explored the precise location of P-GP in the endothelial cells by using an immunoelectron microscopical technique. Fig. 2 shows that the P-GP was confined to the luminal side of the endothelial cells; it was not found on the basal side.

Immunoprecipitation failed to detect P-GP in the endothelial cells because the content of P-GP derived from the endothelial cells of capillary blood vessels is much lower than that of the other non-P-GP proteins in the central nervous system (data not shown).

The present study has shown that P-GP is localized at the luminal side of the endothelial cells of the central nervous system. This raises the following three important points. First, it is already known that the P-GP transports certain anticancer drugs out of cells. If P-GP is present at the luminal side, but not the basal side of the endothelial cells in the central nervous system, this might suggest that P-GP has a physiological role in transporting certain substances into the endothelial cells. It will be necessary to establish an endothelial cell line expressing P-GP to clarify this.

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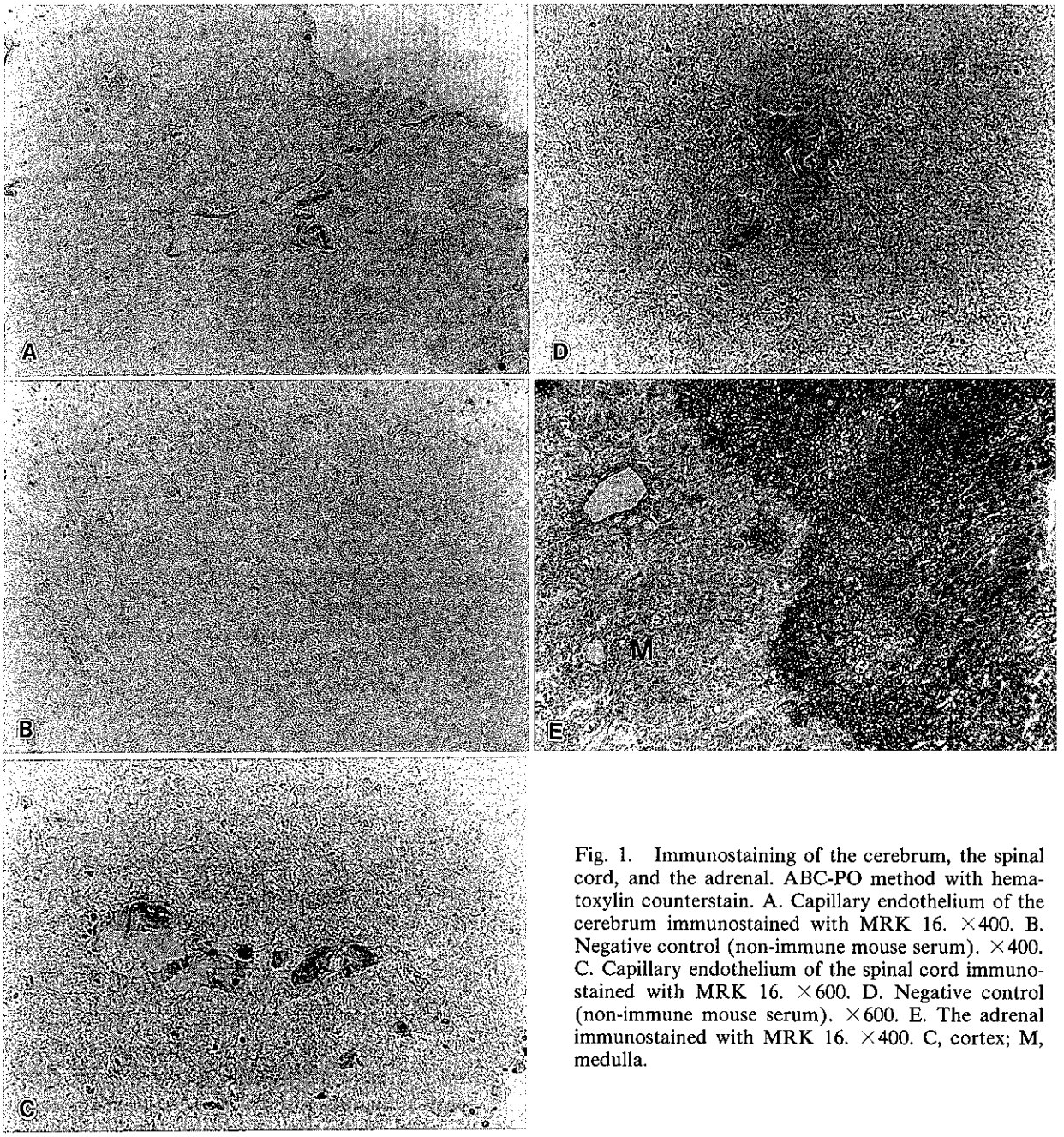

Fig. 1. Immunostaining of the cerebrum, the spinal cord, and the adrenal. ABC-PO method with hematoxylin counterstain. A. Capillary endothelium of the cerebrum immunostained with MRK 16. ×400. B. Negative control (non-immune mouse serum). ×400. C. Capillary endothelium of the spinal cord immunostained with MRK 16. ×600. D. Negative control (non-immune mouse serum). ×600. E. The adrenal immunostained with MRK 16. ×400. C, cortex; M, medulla.

Second, MRK 16 MAb might cross-react with a substance present in the endothelial cells. To rule out this possibility, we utilized an immunoprecipitation technique. No P-GP was immunochemically visualized in the brain and spinal cord (data not shown) because not enough capillary endothelial cell-derived P-GP was extracted. If MRK 16 MAb cross-reacts wit an untargeted substance in endothelial cells, we might expect that the same substance in every other organ will be immunostained with MRK 16 MAb. As P-GP is selectively expressed in the endothelial cells of the central nervous system, this is not the case. Moreover, immunocytochemistry (ABC-Po) is superior in sensitivity to immunoprecipitation.

Finally, it has recently been shown that other monoclonal antibodies against P-GP, C219 and HYB-241, react with endothelial cells in testes as well as in the

Table I. Reactivity of MRK 16 MAb with Various Normal Tissues

Tissue	Reactivities of MRK 16 MAb
Adult adrenal	++ (5/5) cortex
Fetal adrenal	− (0/5)
Placenta	+ (5/5) trophoblasts
Kidney	+ (5/5) proximal tubules
Pancreas	− (0/5)
Rectum	− (0/5)
Gall bladder	− (0/5)
Thyroid	− (0/5)
Pituitary	− (0/3)
Lymph node	− (0/5)
Liver	− (0/5)
Prostate	− (0/5)
Stomach	− (0/5)
Lung	− (0/5)
Submandibular gland	− (0/3)
Mammary gland	− (0/5)
Large intestine	− (0/5)
Small intestine	− (0/5)
Spleen	− (0/3)
Heart	− (0/5)
Skeletal muscle	− (0/5)
Cerebrum	+ (5/5) capillary vessel
Cerebellum	+ (5/5) capillary vessel
Spinal cord	+ (5/5) capillary vessel
Esophagus	− (0/5)
Skin	− (0/5)
Bone marrow	− (0/5)
Testis	− (0/5)

Intensity of immunoperoxidase staining is classified as follows: −, negative; +, positive if less than 50% of the tissue is stained; ++, strongly positive if most of the tissue is stained. Numbers in parentheses indicate number of cases.

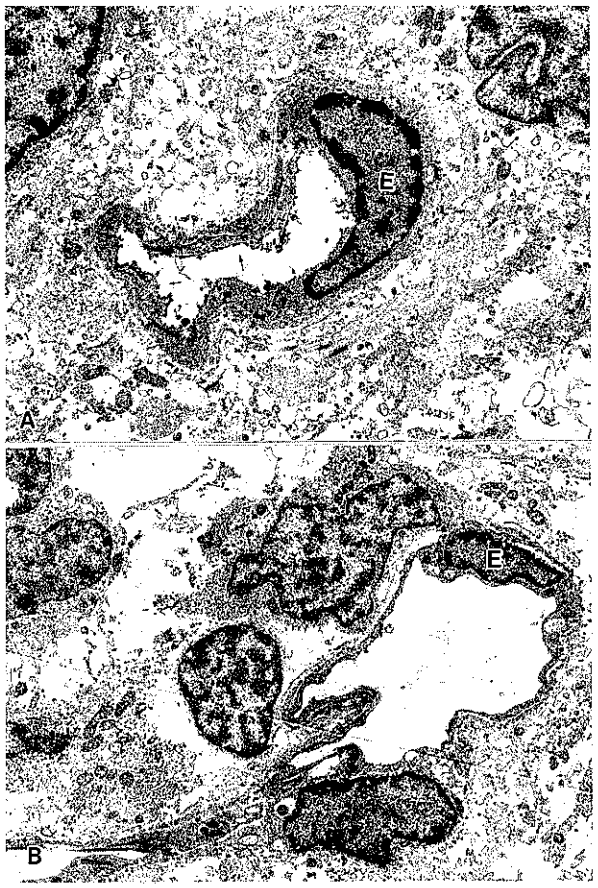

Fig. 2. Immunoelectron micrograph of capillary endothelium of the fetal cerebrum. ABC-PO method. A. MRK 16 (10 μg/ml). × 10,000. The arrow (→) indicates the luminal side of the capillary vessel. E, endothelial cell. B. Non-immune mouse serum (10 μg/ml). × 10,000.

central nervous system. HYB-241 and MRK 16 each recognize an extracellular epitope of P-GP, whereas C219 detects a carboxy-terminal intracellular epitope. This indicates that P-GP may play a physiological role in regulating the entry of certain molecules into the central nervous system and other anatomical compartments in the testes. However, MRK 16 MAb reacted with endothelial cells in the central nervous system,[5] but not with endothelial cells in the testes. We used five different testes and skins, but all the endothelial cells were negative for P-GP. Further study will be required to clarify the discrepancy between our data and Cordon-Cardo's data.

The blood-brain barrier system (BBB) has been a morphological mystery for a long time. The only morphological implication apparent is that the blood-brain barrier is characterized by the presence of intercellular tight junctions between continuous nonfenestrated endothelial cells.[10] Although our finding is interesting, it seems unlikely that the BBB can be simply characterized by the presence of P-GP in the luminal surface of endothelial cells of the central nervous system. In general, lipid-soluble drugs are not excluded by the BBB, but almost all water-soluble drugs including not only P-GP-related drugs but also P-GP-unrelated drugs are affected. Our results may have important implications for the drug design of chemotherapeutic agents for the treatment of infectious diseases and various cancers. Nonetheless, MRK 16 MAb should be used *in vivo* with much caution because there is the possibility that MRK 16 MAb may damage endothelial cells by binding to them.[6]

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