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Abstract

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KEYWORDS: monoclonal antibody, retina, outer segment, immuno-electron microscopy, cell surface antigen

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A Monoclonal Antibody Which Recognizes the Inner and Outer Segments of the Photoreceptor Cells in the Vertebrate Retina

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A monoclonal antibody (MAb-1E7), generated against bovine retinal homogenate, labeled the outer and inner segment layers of the vertebrate retina. Immuno-electron microscopic observation clearly demonstrated that antigen(s) bound by MAb-1E7 was localized in the cell membrane of the outer segment and the distal portion of the inner segment. Western blot analysis revealed that MAb-1E7 recognized 40kD- and 27 kD-polypeptides. Mouse retina with hereditary photoreceptor degeneration (C3H/He and CBA strains) did not involve the MAb-1E7 immunoreactive structures. The present immunocytochemical observation demonstrated that MAb-1E7 was highly specific to the outer segment of the photoreceptor cells and, therefore, can be a useful marker for the cells.

Key words : **monoclonal antibody, retina, outer segment, immuno-electron microscopy, cell surface antigen**

Vertebrate neural retina is composed of five major types of neuronal cells, showing an eight-layered structure (1). Each cell has a characteristic shape and reveals specific responses to light stimuli. Morphological and physiological varieties of the retinal neurons may be related with molecular heterogeneity of each cell. With the advent of hybridoma technology by Köhler and Milstein (2), heterogeneity of chemical components of the vertebrate retina has become apparent by means of monoclonal antibodies (MAbs) (3-8). The photoreceptor cells, rod and cone cells, take the most unique shape in the retina. They constitute the outer and inner segments, perikaryon in the outer granular layer and processes in the outer plexiform layer. There have been numerous studies using MAbs that reacted with particular regions of the photoreceptor cells (3, 4, 6, 9-11). Some antigens recognized by the MAbs were known to be closely related with functions of the cell (6, 10, 11). Thus, MAbs seem to be very useful to discriminatively detect functionally important components of photoreceptor cell. The present study reports that MAb, which was raised against a tissue homogenate of the adult bovine retina, bound to cell-surface molecules of the vertebrate photoreceptor cells.

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Materials and Methods

Preparation of the monoclonal antibody. Adult bovine neural retina was homogenized with phosphate buffered-saline (PBS). BALB/c mice were immunized

with four injections of the retinal homogenate (50 mg wet weight per injection) with Freund's complete adjuvant into foot pads at 4-week intervals. Sixty hours after the final immunization, spleens were removed and splenocytes were fused with PAI myeloma cells (a gift by Dr. Shinobu S. Fujita). Hybridoma cultures were selected and cloned as described previously (2,12). Hybridoma- and clone-culture supernatants were screened by the indirect immunofluorescent method on cryostat sections from the bovine retina fixed by 4% paraformaldehyde (PFA). The MAb reported here, designated as MAb-1E7, was classed as IgG1 as determined by Ouchterlony double diffusion.

Immunohistochemical analysis in retina. For light microscopic (LM) observation, bovine, mouse (C57BL, C3H/He and CBA stains), Wistar rat, quail and frog retinas were used. Bovine and frog retinal tissues were removed from the eye ball and immersed in Zamboni's fixative (4% PFA and 0.5–1% pyclic acid) for 2 h at 4 °C, then soaked in phosphate buffer (PB; 0.1 M, pH 7.4) containing 20% sucrose. The other three animals were perfused through the hearts with Zamboni's fixative under deep anesthesia by sodium pentobarbital. The eyes were removed and immersed in PB containing 20% sucrose. They were cut at 15–20 μm in thickness with cryostat and thaw-mounted onto gelatin-coated glass slides. These sections were sequentially incubated with 5% normal horse serum and with MAb-1E7, and processed according to the ABC method (Vector), then treated with Tris-HCl buffer (50 mM, pH 6.8) containing 0.1% H_2O_2 and 0.05% 3,3′-diaminobenzidine. Each incubation was performed at room temperature and the sections were washed four times with PBS. Some of the sections were incubated with FITC-labeled goat anti-mouse IgG (Cappel) as a secondary antibody.

For the immuno-electron microscopic (EM) observation on the rat retina, specimens were processed as described elsewhere (12). The rat retina fixed by Zamboni's fixative was cut at 60 μm in thickness by cryostat and sections were collected in PBS. The free floating sections were treated with MAb-1E7 and processed according to the ABC method. After color development, sections were re-fixed with 5% glutaraldehyde in PB, then postfixed with 1% osmium tetroxide for 1 h each. They were dehydrated in a graded series of ethanol and embedded in Quetol 812 (Nisshin EM). Ultrathin sections were made and observed under an EM (JEOL, 1200 EX) without stain. The control sections were treated using the same procedures excepting the primary antibody (MAb-1E7).

Western blot analysis. Preparation of retinal lysates, SDS-PAGE of the lysate and transfer of polypeptides from gel to membranes were carried out as described elsewhere (13–15). After blocking of the membranes with 5% nonfat dried milk (Skim Milk, Difco) and 5% normal goat serum, the membranes were incubated with MAb-1E7 for more than 12 h, and subsequently treated with alkaline phosphatase-labeled goat anti-mouse IgG (Stratagene), diluted 1:5000, for 1 h. The membranes were washed in 50 mM Tris-HCl buffer (pH 7.5) containing 0.5 M NaCl and 0.1% Tween 80. After final washing, color was developed by incubating the membrane in 100 mM Tris-HCl (pH 9.5) containing 0.3 mg/ml of Nitro Blue Tetrazolium and 0.15 mg/ml of 5-Bromo-4-Chloro-3-Indolyl Phosphate. Control membranes were treated with supernatants of different kinds of hybridomas secreting the IgG1 monoclonal antibody as a primary antibody.

Results and Discussion

The immunostaining with MAb-1E7 resulted in labeling of the outer segment in the bovine (Fig. 1A), rat (Fig. 1B), C57BL mouse (Fig. 2B) and quail retina (figures not shown). A part of the inner segment seemed to be stained with the MAb, but immunonegative zones were noticed between the outer granular layer and the MAb-1E7 positive zones that were apparently distal parts of the inner segment. Other parts of the retina, including pigment epithelium, were not labeled with the MAb. No labeling was detected in the frog retina as well as in control sections. The C3H/He and CBA mice are known as strains with hereditary degeneration of photoreceptor cells (Fig. 2C). In the retina from two strains MAb-1E7-immunoreactive structures were not encountered (Fig. 2D).

Immuno-EM observations on the rat retina revealed that reaction products were accumulated at the cell membranes of the photoreceptor cells in the outer segment, connecting cilium and the distal portion of the inner segment (Fig. 3). The inner segment near the outer limiting membrane, and perikaryal portion of the photoreceptor cells did not show the MAb-1E7 immunoreactivity.

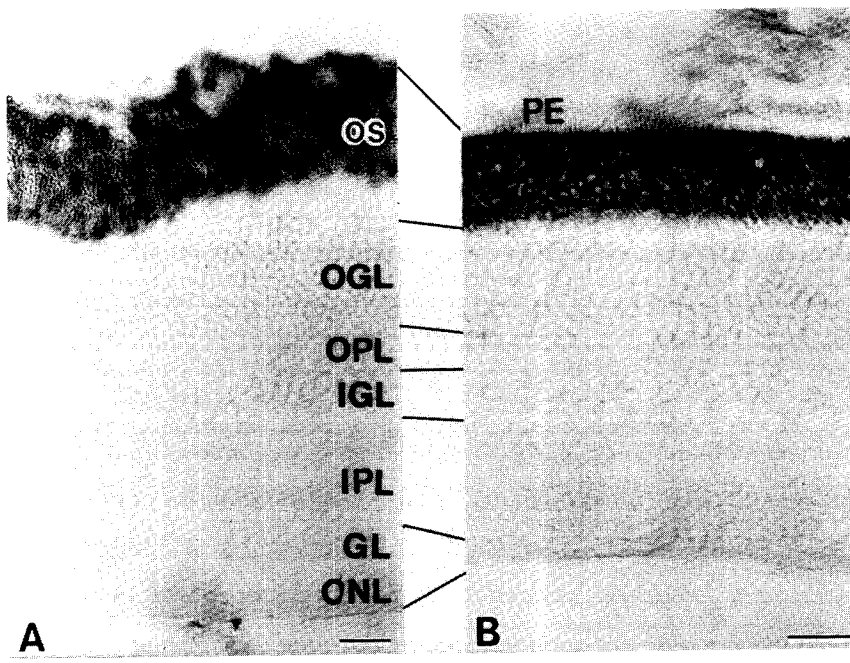

Fig. 1 MAb-1E7 immunohistochemistry with bovine (A) and rat (B) retina, processed according to ABC method. The outer segment (OS) is selectively labeled with the MAb. IGL, inner granular layer; IPL, inner plexiform layer; GL, ganglion cell layer; OGL, outer granular layer; ONL, optic nerve layer; OPL, outer plexiform layer; PE, pigment epithelium; Bars = 20 μm.

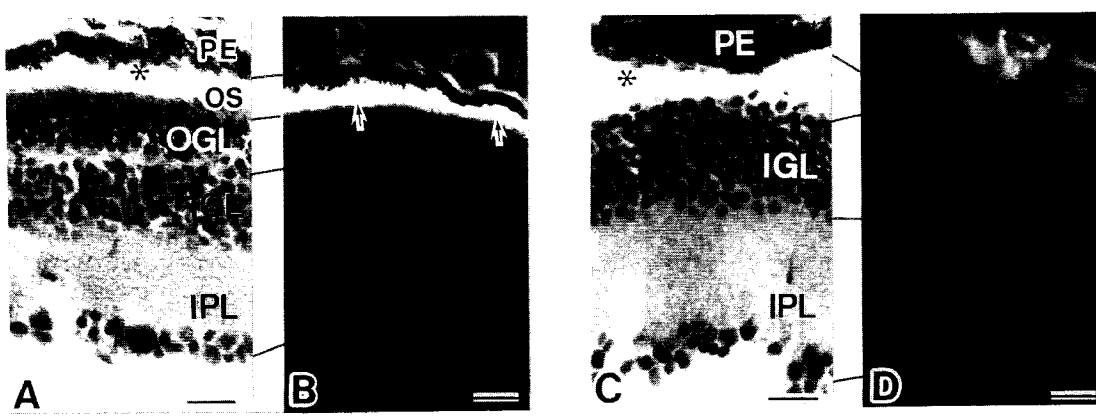

Fig. 2 MAb-1E7 immunohistochemistry with mouse retina, processed by indirect immunofluorescent method (B and D), and corresponding tissues stained by hematoxylin and eosin (A and C). Asterisks in A and C indicate empty space between pigment epithelium and outer segment layer, which is artifactually formed during tissue fixation and sectioning.
A and B, C57BL strain. Outer segment layer (OS) is selectively labeled by the MAb (arrows in B).
C and D, C3H/He strain. Note that retina of this strain lacks the photorecepter cells completely (C) and that no structures labeled with the MAb are involved in the retina (D).
Bars = 20 μm.

No labeling was detected in cytoplasmic regions, including disc membranes within the outer segment. No other retinal cells were immunoreactive to the MAb-1E7.

To determine the molecular weight of antigen(s), Western blot technique was employed. Antigen(s) 1E7 was expressed as two bands corresponding to the molecular weight (MW) about 40kD and 27kD (Fig. 4). The lower band was more intensely labeled by the MAb.

The present study demonstrated the immunohistochemical properties of MAb-1E7 which was generated against the bovine retinal homogenate. MAb-1E7 mainly recognized portions of the outer segments of the vertebrate retina examined.

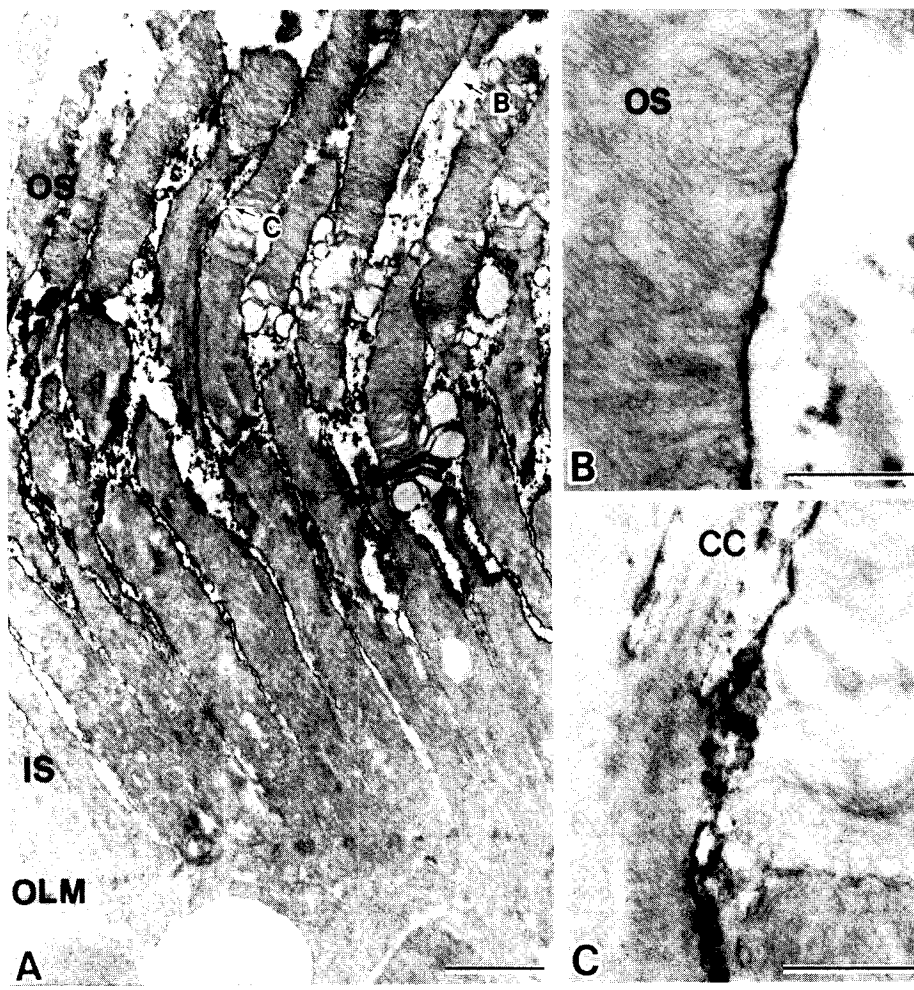

Fig. 3 Electron micrographs of adult rat retina treated with MAb-1E7.
A: Cell membranes of the outer segment (OS) and distal part of the inner segment (IS) show MAb-1E7 immunoreactivity. Areas indicated by arrows B and C are magnified in B and C, respectively. OLM, outer limiting membrane. Bar = 2 μm.
B: High magnification of the outer segment. Note that no immunoreactivity to MAb-1E7 is found on the disc membrane. Bar = 0.5 μm.
C: High magnification of the connecting cilium (CC) whose cell membrane shows MAb-1E7 immunoreactivity. Bar = 0.5 μm.

Immuno-EM observations clearly revealed that antigen(s) bound by the MAb was localized at the cell membrane of the outer segment and distal part of the inner segment. The disc membranes in the outer segment were immunonegative to MAb-1E7. It is well-known that, in the outer segment layer, processes of the pigment epithelial cells are intercalated with the distal portion of the photoreceptor outer segment (16). Therefore, immunohistochemical examination was also performed using C3H/He and CBA mouse retina whose photoreceptor cells were completely degenerated at adult stages, in order to deny a possibility that MAb-1E7 stained the processes of the pigment cells. Immunoreactive structures were not detected in the retina of these two strains. Therefore, our present findings clearly revealed that MAb-1E7 was highly specific to the membrane polypeptides of the outer segment and distal portion of the inner segment, and, that it can be a useful marker for the photoreceptor cells.

The photoreceptor cells of the vertebrate retina have three compartments, and hybridoma technology elucidates molecular diversity of the compartments (3). MAbs have been reported to be specific to the photoreceptor cell, and some of them bind selectively to a particular compartment (3,4). Although some MAbs specific to the outer segment were reported to bind to rhodopsin (MW of 39kD) (6,11) or alpha-transducin (MW of 39 kD) (10), the chemical nature of antigens recognized by other outer segment-specific MAbs were still unknown (5). Lectin histochemistry also demonstrated the photoreceptor cell-specific carbohydrates (9). It is, therefore, reasonable to suggest that many antigens may be involved in the outer segment of photoreceptor cells. MAb-1E7 of the present study also bound to one and/or some of these antigens with MW of 40kD and 27kD. Membrane-specific morphological property of MAb-1E7 was similar to rhodopsin monoclonal antibody, RHO-C7 (5), but MW of antigen 1E7 was different from rhodopsin. Therefore, it is probable that MAb-1E7 recognized novel antigens in the retina.

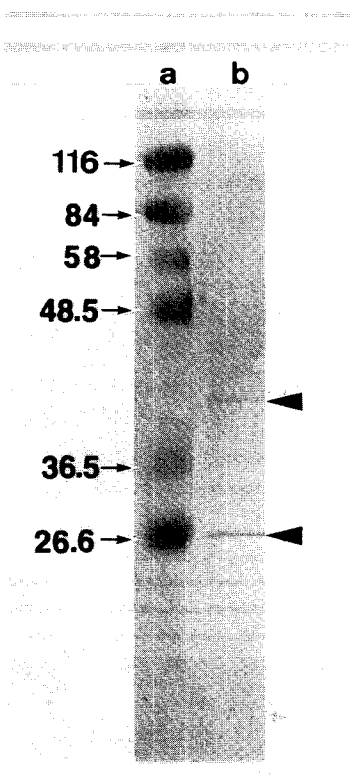

Fig. 4 Western blot analysis of the retinal polypeptides from adult rat with MAb-1E7 (lane b). The polypeptides are separated in 10 % uniform gel. Molecular weights of polypeptides immunostaied with the MAb correspond to 40kD and 27kD (arrowheads). 27kD-polypeptide was more intensely stained. Lane a; Molecular weight markers. Numbers on the left column indicate the molecular weight (kD) of polypeptides.

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