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A characteristic protein highly expressed in guinea pig inner ear, defined by monoclonal antibody WH-1

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Abstract

A new monoclonal antibody (termed WH-1; isotype IgG_{2b}) was established using a homogenate of dissected guinea pig cochleas ($N = 60$) as immunogen. Western blotting and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) identified the WH-1 antigen as a protein or glycoprotein with $M_r \approx 40$ kDa. Immunoperoxidase treatment of histologic cryosections of guinea pig cochlea, followed by light microscopic examination, revealed strong positive staining at three sites: (i) parts of Hensen's stripe, marginal band, covering net, and Kimura's membrane (within the tectorial membrane [TM]); (ii) Deiters' cells, pillar cells, Hensen's cells, and stereocilia of outer hair cells (within the organ of Corti); (iii) interdental cells, inner and outer sulcus cells, Reissner's membrane, and surface membrane of stria vascularis epithelium. Similar staining patterns were observed for cryosections of rat and mouse cochleas. Only a trace quantity of cross-reacting protein was detectable in brainstem. The protein was not detectable in tongue extract by Western blotting. However, sections of brainstem and tongue did show positive immunohistological staining with WH-1. Localization of WH-1 antigen was further examined by electron microscopy. WH-1 positivity on outer hair cell stereocilia, certain sites on the TM, interdental cell surface, Reissner's membrane epithelia, and inner and outer sulcus cells was confirmed. WH-1 antigen was not detected on inner hair cell stereocilia by light or electron microscopy. The localization of WH-1 antigen on outer hair cell stereocilia and TM suggests that it may play some role in adhesion between these structures.

Key words: Guinea pig cochlea; Tectorial membrane; Outer hair stereocilia; Kimura's membrane; Western blotting; 40 kDa Protein

1. Introduction

The monoclonal antibody (mAb) approach (Köhler and Milstein, 1976) has been successfully applied for characterization of specific molecules which play key roles in differentiation and development (Hakomori and Kannagi, 1983; Kennett et al., 1984; Feizi, 1985; Fenderson et al., 1990), and also for identification of tumor-associated antigens (Hakomori, 1984; 1989). In the present study, we attempted to use the mAb approach for detection of characteristic functional molecules in the mammalian inner ear. The existence of such molecules in the organ of Corti has long been suspected. However, because of its inaccessibility and the minute amount of harvestable tissue, unambiguous identification of molecules specific to the organ of Corti has been difficult (Thalmann et al., 1980; Zajic et al., 1991; Ptok et al., 1991). Many investigators have reported the presence of glycoconjugates in the inner ear using lectin histochemical techniques (Gil-Loyzaga et al., 1985; Tachibana et al., 1987; Santi and Anderson, 1987; Rueda and Lim, 1988). The presence of specific proteins, particularly adhesive proteins of the inner ear, has also been described, and the proposed functions of these proteins have received considerable attention (Thalmann et al., 1980; 1990; Oberholtzer et al., 1986; Richardson et al., 1987b; 1987a; 1990). In the present study, a mAb (WH-1) was established by immunization of Balb/c mice with a homogenate of

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guinea pig cochleas. WH-1 was shown to stain a protein or glycoprotein ($M_r \approx 40$ kDa) localized at the tectorial membrane (TM) and organ of Corti of guinea pigs, and also to label neuron subpopulations in auditory and other nuclei of guinea pig brainstem.

2. Materials and methods

2.1. Antigen preparation and immunization

Thirty albino guinea pigs (250–300 g) were sacrificed by CO_2 inhalation. Temporal bones were harvested and immediately placed in phosphate-buffered saline (PBS) at 4°C. Using microdissection, the TM and organ of Corti from each temporal bone ($N = 60$) were isolated. Tissue was pooled in 1 ml PBS, minced with microscissors, and sonicated for 1 h at 4°C. Antigen was prepared by adding the sonicated portion to 1 ml RIBI adjuvant as per manufacturer's instructions (RIBI Immunochemical Research, Hamilton, MT).

Six-week-old Balb/c mice were immunized using the antigen described above. Animals were given four intraperitoneal injections at three-week intervals.

2.2. Fusion

Three days after the final injection, immunized mice were sacrificed, spleens removed and washed with RPMI-1640 (Irvine Scientific, Santa Ana, CA) 2 × for 5 min. Cell collectors were used to obtain cell suspensions; cells were then washed with RPMI 2 × for 5 min, pelleted, suspended in 40 ml RPMI, and counted. NS-1 hybridoma cells (Köhler and Milstein, 1976) were conditioned in rapid-growth phase, harvested, washed 2 × with RPMI, and counted. NS-1 cells were combined with spleen cells in a 1:5 ratio. The subsequent procedure can be summarized as follows: centrifuge (200 × g, 10 min); remove supernatant as quickly as possible; loosen cell pellet by tapping gently; warm to 37°C for more than 5 min; add 50% polyethylene glycol 1500 (Boehringer Mannheim Biochemicals, Indianapolis, IN) in RPMI prewarmed to 37°C; incubate 45 s; add 1 ml RPMI over 1 min; add another 1 ml RPMI over 1 min; add 7 ml RPMI over 3 min; add 10 ml RPMI; centrifuge (150 × g, 7 min) and get pellet; add 20 ml RPMI including 15% fetal calf serum glutamine, pipette gently and transfer to 10 cm Petri dish; incubate 30 min at 37°C; add 37.8 ml RPMI supplemented with 15% fetal calf serum, 2 mM L-glutamine (Sigma Chemical Co., St. Louis, MO), 100 mM hypoxanthine (Sigma), 0.4 mM aminopterin (Sigma), and 16 mM thymidine (Sigma) containing thymocytes as feeder cells; add aliquots (200 μl/well) to 96-well microtiter plates; incubate at 37°C; exchange 100 μl of medium on days 4, 7, and 10; determine immunoreactivity of supernatant on day 10.

2.3. Screening

Hybridoma supernatant (100 μl) was collected 10 days after fusion and tested against 10 μm cryostat sections of guinea pig cochlea fixed by intralabyrinthine perfusion of periodatelysine paraformaldehyde (PLP) (McLean and Nakane, 1974) and prepared for light microscopic examination as described below. Hybridoma cells exhibiting positive activity were prepared by limiting dilution method and replated in a 96-well microtiter plate at one cell per well. Hybridoma supernatant was reexamined after 10 days incubation, and the dilution method repeated. Limiting dilution and replating of hybridoma cells were performed at least 2 × prior to collection of mAb.

2.4. Isotype determination

Supernatants of hybridoma cells which demonstrated immunoreactivity with guinea pig cochlea were tested for isotype by enzyme-linked immunostaining assay. Briefly, 96-well microtiter plates were precoated with goat anti-mouse immunoglobulin and incubated for 1 h at 37°C with the positive hybridoma supernatants. Plates were washed with PBS and subsequently incubated for 1 h with IgG, IgG_1, IgG_3, IgM (Fisher Scientific, Pittsburgh, PA), IgG_{2a}, and IgG_{2b} (Southern Biotechnology, Birmingham, AL) (all horseradish peroxidase-labeled, goat anti-mouse immunoglobulins). Spectrophotometric absorbance was determined after reaction with the substrate 2,2,-azinobis-3,-ethylbenzthiazoline sulfonic acid (Porstmann, 1981).

2.5. Immunohistochemical examination of cochlea by light microscopy

Guinea pig cochleas were removed immediately following decapitation under anesthesia (CO_2 inhalation), and perfused with 2% PLP (McLean and Nakane, 1974) via perilymphatic space under a dissecting microscope. Perfusion was followed by immersion in fresh PLP for 4 h at room temp. Cochleas were then immersed in PBS for 2 h at room temp, and decalcified by immersion in 0.1 M ethylenediaminetetraacetic acid (EDTA) for 1 wk at 4°C. During this period, EDTA solution was changed every two days. Next, cochleas were washed in PBS for 2 hr, and placed in PBS at 4°C with increasing concentration of sucrose: at 2-h intervals, sucrose concentration was changed from 5% to 10%, 15%, 20%, and finally 30%. Cochleas were rapidly frozen in OCT embedding compound (Miles Scientific, Kankakee, IL) in a liquid N_2-cooled heptane bath.

Frozen sections (10 μm) were cut on a cryostat and mounted on gelatin-coated slides. Slides were dried, stored at −80°C, and tested for immunoreactivity against mAb within one month of fixation.

Frozen sections were allowed to thaw and air dry at room temp for 1 h prior to immunostaining. Then, sections were immersed in acetone (10 min, 4°C), washed in PBS (10 min, 3 ×), blocked with 4% normal horse serum in PBS (30–60 min, room temp), incubated in primary antibody WH-1 (overnight, 4°C), washed in PBS (10 min, 3 ×), incubated in secondary antibody (horse anti-mouse Ig 1:200 in PBS) (1 h, room temp), washed in PBS (10 min, 3 ×), combined with avidin-biotin complex (ABC) (1 hr, room temp), washed in PBS (10 min, 3 ×), incubated with 3,3-diaminobenzidine (2–10 min, room temp), and washed in distilled water (10 min, 3 ×). Control sections were treated in the same manner except that primary antibody was replaced by NS-1 supernatant plus irrelevant hybridoma supernatant. After the above procedures, sections were dehydrated in ethanol, immersed in xylene, and placed under coverslips for light microscopic examination. Pictures were taken using an Olympus microscope equipped with Nomarski's differential interference apparatus.

2.6. *Immunocytochemical examination of cochlea by electron microscopy (EM)*

Guinea pig cochleas ($N = 5$) were fixed by intralabyrinthine perfusion of PLP, held for 4 h in PLP (4°C), washed, and microdissected to open all turns. Blocking was performed by immersing cochleas for 1 h in 4% normal horse serum in PBS containing 0.5% bovine serum albumin and 0.01% Triton X-100 at 4°C. Cochleas were then incubated overnight at 4°C in mAb

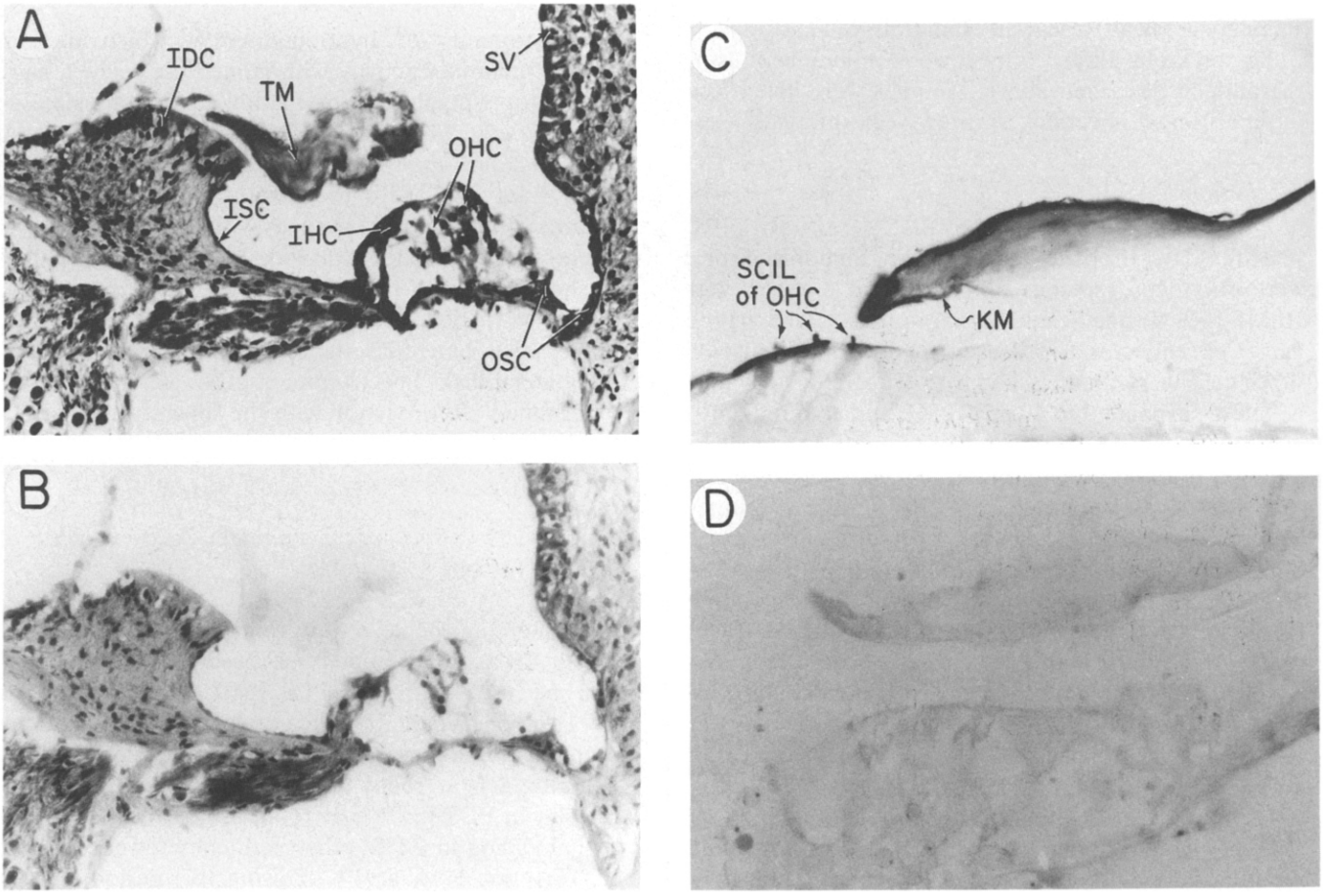

Fig. 1. WH-1 positivity in guinea pig cochlea. (A), WH-1 positive staining of cochlear duct with hematoxylin counterstaining, ×300. IDC, interdental cell; SV, stria vascularis; ISC, inner sulcus cell; OSC, outer sulcus cell; IHC; inner hair cell; OHC, outer hair cell. Note strong positivity of TM, IDC, ISC, OSC, apical surface of strial marginal cells, Deiters' cells, pillar cells, and cuticular plate of OHC. (B), negative control of A with hematoxylin counterstaining. In this control study, WH-1 primary antibody was replaced by NS-1 cell culture supernatant. As contrasted with this panel, WH-1 immunoreactivities in panel A are quite evident. (C), WH-1 staining of TM and organ of Corti surface without hematoxylin staining, ×750 (high power). SCIL of OHC, stereocilia of outer hair cells; KM, Kimura's membrane. Note strong positivity of covering net, fibrous layer, lymbal layer, and KM in TM, as well as cuticular plate of OHC, SCIL of OHC, outer phalangeal cells, and Hensen's cells. (D), negative control of C. These pictures are from the same specimen used for EM examination (Fig. 3).

or control hybridoma supernatant on a shaker table. Following three washes in PBS containing 0.1% bovine serum albumin and 0.01% Triton X-100, cochleas were incubated in 1:200 dilution of biotinylated horse anti-mouse IgG (2 hr), and routine ABC immunoperoxidase staining was performed using an Elite ABC kit (Vector Labs, Burlingame, CA) as per manufacturer's instructions. After three washes with PBS, post-fixation was performed in 1% osmium tetroxide in PBS for 1 h at 4°C. Cochleas were dehydrated through graded ethanol, followed by infiltration and embedding in Spurr's epoxy resin. A rotary diamond saw was utilized to make mid-modiolar cuts and expose cochlear turns for cross-sections through the organ of Corti. Semi-thin and ultra-thin sections were made with a Sorvall MT-2b ultramicrotome, and sections were contrasted with uranyl acetate and lead citrate prior to EM examination in a Philips EM-410.

2.7. *Immunohistological examination of control tissues*

Heavily anesthetized guinea pigs were perfused transcardially with PLP, and various tissues were harvested for snap freezing and cryosectioning. Specimens of forebrain, brainstem, cerebellum, olfactory bulb, retina, liver, kidney, intestine, lung, heart, muscle, spleen, and thyroid gland were immersed in PLP and fixed for 4–12 h at room temp. Tissues were washed in PBS for 2 h, cryoprotected with increasing concentrations of sucrose in PBS, held in 30% sucrose/PBS overnight at 4°C, and rapidly frozen in OCT embedding compound in a liquid N_2-cooled heptane bath. Frozen sections (10 μm) were cut and mounted on gelatin-coated slides. Slides were stored at −80°C and tested for immunoreactivity against mAb within one month of fixation. Frozen sections were treated identically to light microscopic sections of cochlea prior to immunostaining. Positive and negative hybridoma supernatants were tested for immunoreactivity against these control tissues using the immunostaining technique previously described for screening. Following immunostaining, slides were lightly stained with hematoxylin or thionine (Sigma), dehydrated through graded ethanols, cleared with xylene, and cover-slipped. Rat and mouse cochleas were similarly fixed, frozen, cryosectioned, and tested for immunoreactivity along with guinea pig control tissues.

2.8. *Identification of WH-1 antigen by Western immunoblotting*

Initially, in order to determine whether the isolated antigen was glycolipid or glycoprotein, the reactivity of positive tissue sections was tested on slides treated with 0.05% trypsin (Irvine Scientific) (6 h, 37°C), or isopropanol-hexane-water (IHW) (55:25:20) (6 h, 37°C) (Hakomori, 1983). Positive tissues (brainstem, retina, tongue, and inner ear) were solubilized in 2 ml lysis buffer (0.5% Nonidet P-40 (Sigma), 10 mM Trizma Base (Sigma), 150 mM NaCl, 5 mM EDTA, pH 7.4) containing protease inhibitors (including 20 μl phenylmethyl sulfonylfluoride, 20 μl aporotin, and 4 μl leupeptin [Sigma]). Tissues were finely minced (4 mm^3) using microscissors, and homogenized using a Wheaton A homogenizer in ice bath. Homogenates were centrifuged ($5500 \times g$) for 15 min at 4°C and protein quantitation was performed on the supernatants (Lowry et al., 1951). SDS-PAGE was performed as previously described (Laemmli, 1970). Supernatants of tissue homogenates and M_r standards (Amersham Corp., Arlington Heights, IL) were mixed with loading buffer (50 mM Tris-Cl, pH 6.8, 2% SDS, 10% glycerol, 0.005% bromophenol blue) to achieve a concentration of 20–200 μg protein/sample/lane, and loaded onto a 4–

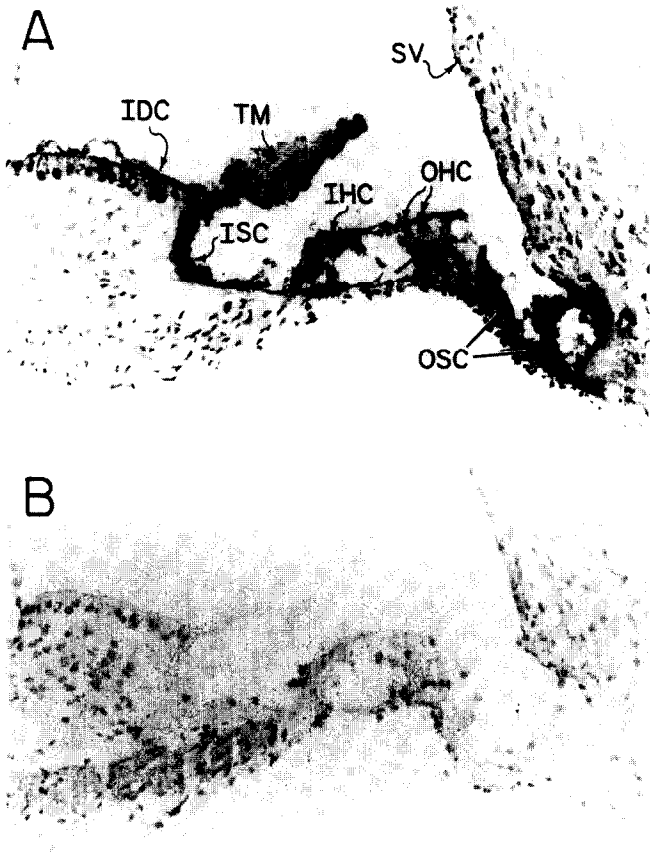

Fig. 2. WH-1 positivity in rat cochlea. (A), WH-1 positive staining of cochlear duct with hematoxylin counterstaining, ×200. Abbreviations as in Fig. 1. (B), negative control of A (WH-1 primary antibody replaced by NS-1 culture supernatant) with hematoxylin counterstaining. Although staining intensity in rat cochlea was weaker than in guinea pig cochlea, distribution of WH-1 immunoreactivity was quite similar. Note positivity of TM, IDC, ISC, OSC, apical surface of strial marginal cells, Deiters' cells, pillar cells, and cuticular plate of OHC.

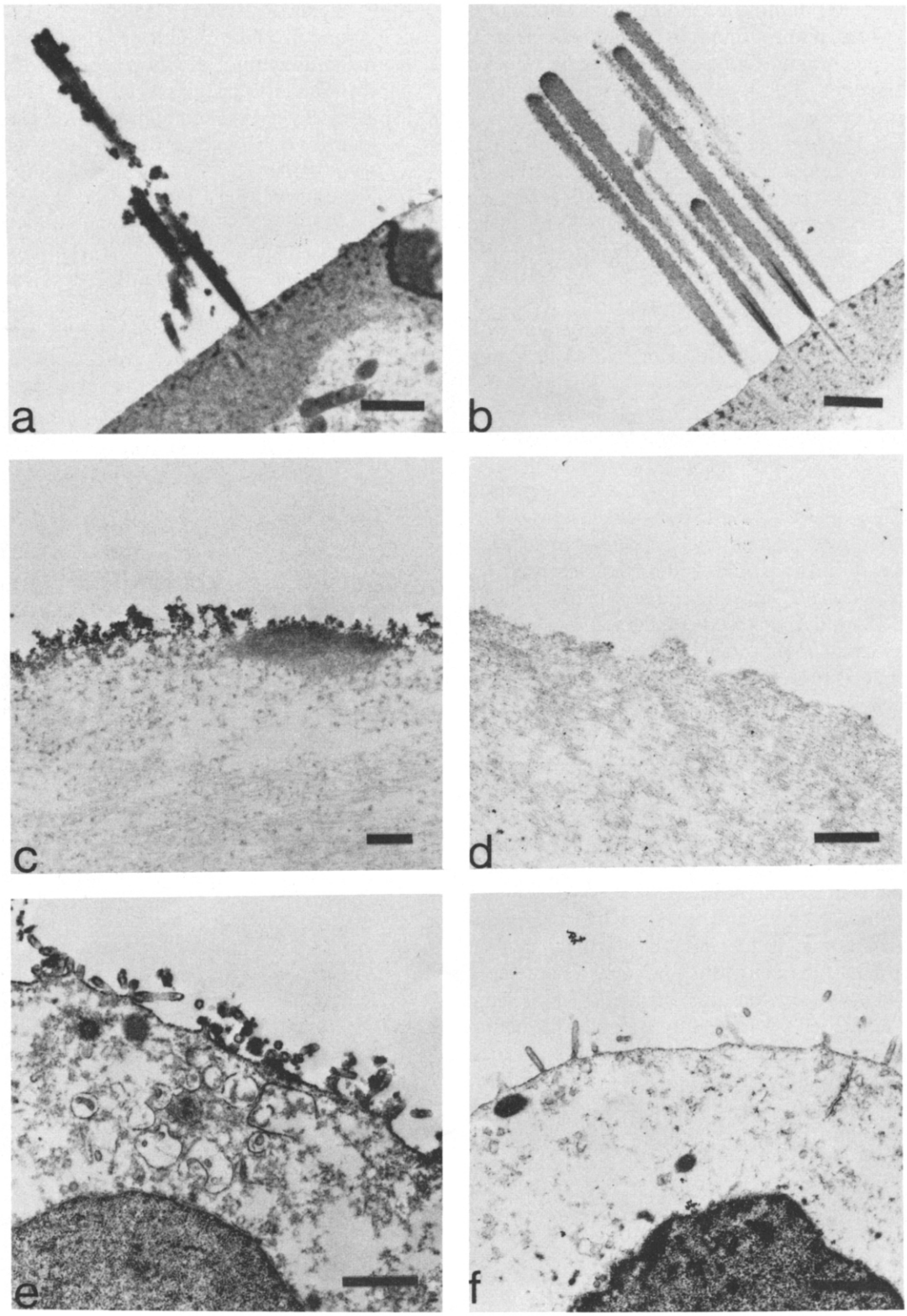

Fig. 3. WH-1 positivity in guinea pig cochlea by EM. (A), WH-1 positive staining of outer hair cell stereocilia. (C), WH-1 staining of TM surface facing outer hair cell stereocilia. (E), WH-1 staining of interdental cell surface. (G), WH-1 staining of scala media surface of Reissner's membrane. SV, scala vestibulae; SM, scala media. (I), inner hair cell stereocilia treated with WH-1; no staining is seen. (B, D, F, H) are negative controls for (A, C, E, G) respectively. Negative control for (I) not shown. Bar represents 1 μm for (A, B, C, D, F, I); 2 μm for (G, H).

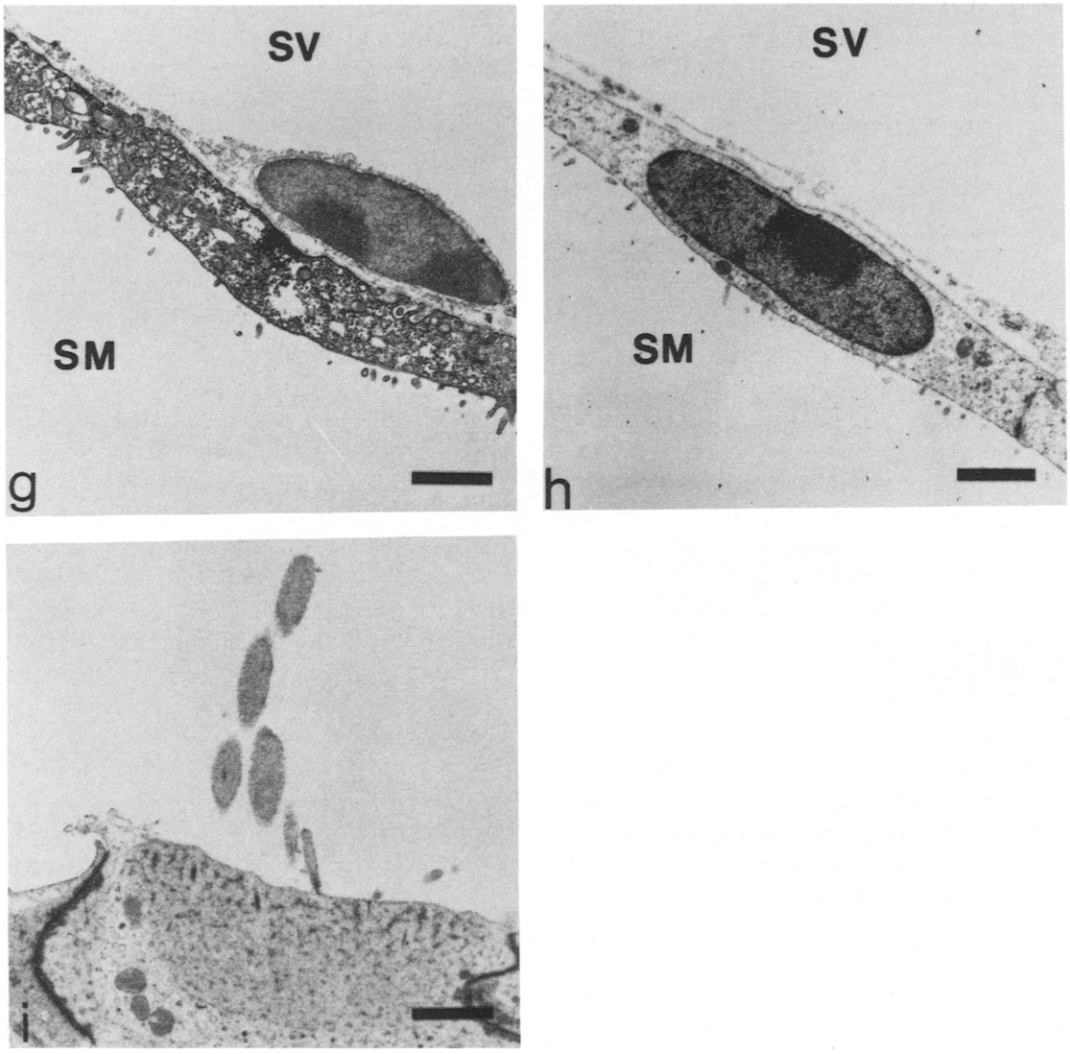

Fig. 3 (continued).

12% Tris-glycine gel (Novex, Encinitas, CA). All post-extraction procedures were performed within 1 hr.

Following electrophoretic separation, proteins were transferred from the gel onto a nitrocellulose membrane (Bio-Rad Laboratories, Richmond, CA) by electrophoresis. The nitrocellulose membrane was incubated for 1 h in a blocking buffer consisting of 5% non-fat dry milk (Carnation) in PBS. Nitrocellulose membranes were then reacted with positive supernatant of the antibody-producing hybridoma. After washing with PBS (3 ×, 5 min), the membranes were incubated for 1 h in 1:1000 dilution of goat anti-mouse Ig (Fisher Scientific). Following another wash, membranes were developed using Enhanced Chemiluminescence Western blotting kit (Amersham) as per manufacturer's instructions.

2.9. Animal welfare

The care and use of the animals reported on in this study were approved by the Animal Welfare Committee of The Biomembrane Institute, which follows the guidelines of the U.S. National Institutes of Health.

3. Results

Originally, 13 hybridoma supernatants showed positive immunostaining when tested on cryostat sections of guinea pig cochlea. Five of these clones were maintained, and 3 clones with relatively strong antibody activity were established. Two of these 3 showed only slight positive immunohistological staining of cochlear nerve. The third clone showed strong, consistent staining of specific regions of the cochlea, and was therefore chosen for further characterization. The mAb, named WH-1, was shown to have isotype IgG_{2b}.

3.1. Localization of WH-1 antigen

Fig. 1 shows positive immunoreactivity of WH-1 when reacted with a 10 μm cryosection of guinea pig

cochlea. Staining with WH-1 (with hematoxylin counterstaining) and a negative control (without WH-1) are shown in Panels A and B respectively. WH-1 staining pattern focused on TM, particularly Kimura's membrane (Kimura, 1966; Lim, 1986), and a negative control are shown in Panels C and D, respectively. The strongest staining was observed at the inner and outer sulcus cells. Interdental cells were also strongly stained, and Hensen's stripe (in part), marginal band, covering net, and Kimura's membrane (in part) were positive on TM. In the organ of Corti, Deiters' cells, pillar cells, Hensen's cells, and outer hair cell stereocilia were positive. Reissner's membrane was positive. Some staining was also evident on the surface membrane of stria vascularis epithelium.

WH-1 was tested for immunoreactivity with frozen sections of various other guinea pig tissues; positive staining was observed for several neuronal populations in the brainstem, retinal ganglion cells, and tongue muscle cells (data not shown). Interestingly, some of the strongest staining was observed in subpopulations of neurons in the auditory nuclei. In the medial superior olivary nucleus, neuron somata and primary dendrites were stained. In the anteroventral cochlear nucleus, granular cells showed no staining, but other neurons were stained. Positive immunoreactivity with WH-1 was also observed in neurons located in the medial nucleus of the trapezoid body, lateral superior olivary nucleus, and vestibular nuclei. Although staining in these other neuronal tissues was clearly observable, its intensity as compared to that in cochlea was weak. Chemical quantity of WH-1 antigen in these other tissues, as analyzed by Western immunoblotting (see following section), was 500- to 1000-fold less than that in cochlea. Neuronal staining was not limited to auditory nuclei. Dispersed groups of neurons were stained, but some populations were completely negative. For example, no staining was seen in Purkinje's or granular cells of the cerebellum. Other tissues tested and not mentioned above were also negative.

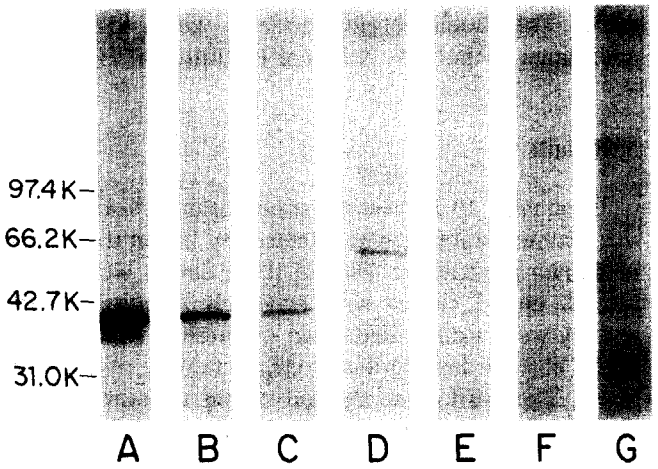

Fig. 4. Detection of WH-1 antigen by Western immunoblotting. Each lane received 20 μl of supernatant from tissue homogenate. (Lane A), cochlea extract; protein concentration [p.c.] 4.10 mg/ml. (B), cochlea extract; p.c. 0.41 mg/ml. (C), brainstem extract; p.c. 10.2 mg/ml. (D), tongue extract; p.c. 0.85 mg/ml. (E), retina extract; p.c. 0.91 mg/ml. (F), liver extract; p.c. 1.02 mg/ml. (G), lung extract; p.c. 1.04 mg/ml.

Staining pattern of a frozen section of rat cochlea by WH-1 with hematoxylin counterstaining is shown in Fig. 2A (negative control without WH-1 is shown in Fig. 2B). The pattern is similar to that of guinea pig cochlea. Staining was decreased or absent when sections were pretreated with trypsin. Staining pattern and intensity were not affected when sections were pretreated with IHW.

EM examination of tissues reacted with WH-1 (Fig. 3) revealed staining of the stereocilia of outer hair cells (Panel A), TM (Panel C), interdental cell surfaces (Panel E), epithelial cells of Reissner's membrane (Panel G), and inner and outer sulcus cells (not shown). Stereocilia of inner hair cells were unstained. Panels B, D, F, and H are negative controls for Panels A, C, E, and G, respectively.

3.2. *Western immunoblotting*

The ability of WH-1 to detect its epitope on Western blots of detergent-extracted and SDS-PAGE-separated proteins was examined. Liver and lung preparations were used as negative controls. WH-1 reacted with proteins from cochlea, brainstem, and tongue blots (Fig. 4). No bands were detectable on blots from retina, liver, or lung. A clear band at ≈ 40 kDa was observed on Western blots from cochlea and brainstem. From tongue blot, a clear band of slightly higher M_r (≈ 50 kDa) was observed. Results from Western blots of gels electrophoresed in both reducing and non-reducing conditions (i.e., presence or absence of 2-mercaptoethanol) were the same. The WH-1 epitope had a much higher concentration in cochlea than in brainstem.

4. Discussion

Specific molecules essential for the function of specific types of cells and organelles are common in living organisms. Well-known examples include hemoglobin (a transporter of O_2) in erythrocytes, myosin/actin/tropomyosin complex in myofibrils, retinin (carried by rhodopsin) as a phototransmitter in retinal cells, and chlorophiles as photoenergy transformers in chloroplasts of plant leaves. Many workers have attempted to identify specific functional molecules in the mammalian inner ear. We have previously demonstrated the

presence of specific carbohydrate structures (Le^x [Galβ1 → 4[Fucα1 → 3]GlcNAcβ1 → R], Le^y [Fucα1 → 2Galβ1 → 4[Fucα1 → 3]GlcNAcβ1 → R], sialosyl-Le^x) in the guinea pig TM and organ of Corti, and suggested the involvement of these structures in inner ear development and sound wave → nerve impulse conversion (Hozawa et al., 1993). However, this study was based on already-established mAbs directed to SLe^x, Le^x, Le^y, and other carbohydrates. In the present study, a new mAb (WH-1) was established against guinea pig inner ear homogenate as immunogen. The epitope of WH-1 was shown to be a specific macromolecule localized at certain sites in the guinea pig, particularly the organ of Corti, cochlear duct, and brainstem. Much weaker expression of a similar epitope was observed for retina and tongue, but the tongue epitope had a different M_r value. Using Western immunoblotting technique, a strong band with M_r 40 kDa was observed from preparations of cochlea, even when 20 μl of cochlea extract containing 0.41 mg/ml protein was analyzed. A faint band with similar M_r was detected from brainstem, even when 25 times more protein was applied. A positive band at $\approx$ 50 kDa was observed in a Western blot of tongue. No band was observed on Western blots of retina or control tissues (lung and liver). If the antigen recognized by WH-1 is, in fact, a glycoprotein, variability in the carbohydrate moiety could account for the higher M_r band seen on the Western blot of tongue. Absence of staining on Western blot of retina suggests that this antigen may be present in retina only in very low concentrations. Pretreatment of tissue sections with IHW had no effect on WH-1 immunostaining. For these reasons, and because positive bands were observed following Western blotting, we conclude that the antigen recognized by WH-1 is a protein or glycoprotein.

Proteins specific to the mammalian organ of Corti (OCP-I and OCP-II) were previously described (Thalmann et al., 1980). A series of mAbs (KHRI-1 through -5) specific to guinea pig cochlea were recently reported (Zajic et al., 1991; Ptok et al., 1991). We assume that the antigen recognized by mAb WH-1 is different from these proteins, because of differences in M_r and immunocytochemical localization.

Although the high concentration of WH-1 antigen in the inner ear suggests that this protein must have a functional role, the distribution pattern of immunoreactivity gives little hint of what this role might be. The antigen is also present in brainstem, retina, and tongue, but at much lower concentrations. Based on the intensities of Western blotting bands, and the quantities of protein extracted from each tissue assayed, we judged that the concentration of WH-1 antigen in cochlea is at least 500 times higher than that in brainstem, retina, or tongue. Interdental cells, which are thought to be secretory cells in the cochlear duct (Thorn et al., 1979), show strong WH-1 staining.

One of our most interesting findings is that stereocilia of outer hair cells show positive WH-1 staining, while stereocilia of inner hair cells do not. Takasaka et al. (1983) reported that a gap exists between TM and stereocilia of the inner hair cells, whereas the stereocilia of outer hair cells are linked to TM. Therefore, it is possible that WH-1 antigen plays a role in attachment of outer hair cell stereocilia to TM.

A slightly different immunostaining pattern was observed for some structures by EM as compared to cryostat sections. In EM, interdental cells showed only surface membrane staining, and intensity of staining on TM was also very superficial. This result may be due to poor penetration of WH-1 by the pre-embedding technique which we used for EM immunocytochemistry.

The functional role of the WH-1 antigen is unclear at this time. However, mAbs such as WH-1 are obviously useful for obtaining more information about molecules specific to the inner ear, and their possible functions. Cloning of cDNA encoding WH-1 antigen, and sequencing of the cDNA clone, will permit characterization of the properties of WH-1 antigen. A homology search of already-characterized proteins may provide clues to the function of WH-1 antigen. In vivo studies performed with WH-1 and similar mAbs should give information about the functional roles of this and other molecules highly expressed in the cochlea.

Acknowledgments

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