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SHORT COMMUNICATION

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# Elongated pericyte-like cells connect discrete capillaries in the cochlear stria vascularis of gerbils and rats

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**Abstract** Bridging structures between discrete capillaries in the stria vascularis of the cochlea were studied morphologically in gerbils and rats. Serial thin sections for transmission electron microscopy revealed (1) that elongated cells surrounded by the basal lamina provided the structural basis for the bridging structure, (2) that the basal lamina surrounding the elongated cell extended to the basal lamina around the capillary endothelial cell, (3) that the electron density of the cytoplasm was similar to that of the pericytes around the capillaries, and (4) that the cell was attached to the capillaries at both ends only. Visualization of the basal lamina by immunofluorescent methods revealed (1) that capillaries were often bent at the site of attachment of the bridging cell, (2) that the bridging cell bifurcated occasionally, and (3) that the density of the bridging cell was much higher in the stria vascularis than in the underlying spiral ligament. Filamentous actin visualized by fluorescent phalloidin was not apparent in the bridging cell. We propose that the bridging cell provides mechanical strength to the tortuous capillary network in the stria vascularis and participates in the specific function of the stria vascularis in cooperation with other types of cells.

**Key words** Basal lamina · Immunohistochemistry · Confocal laser microscopy · Cochlea · Mongolian gerbil · Rat (Wistar)

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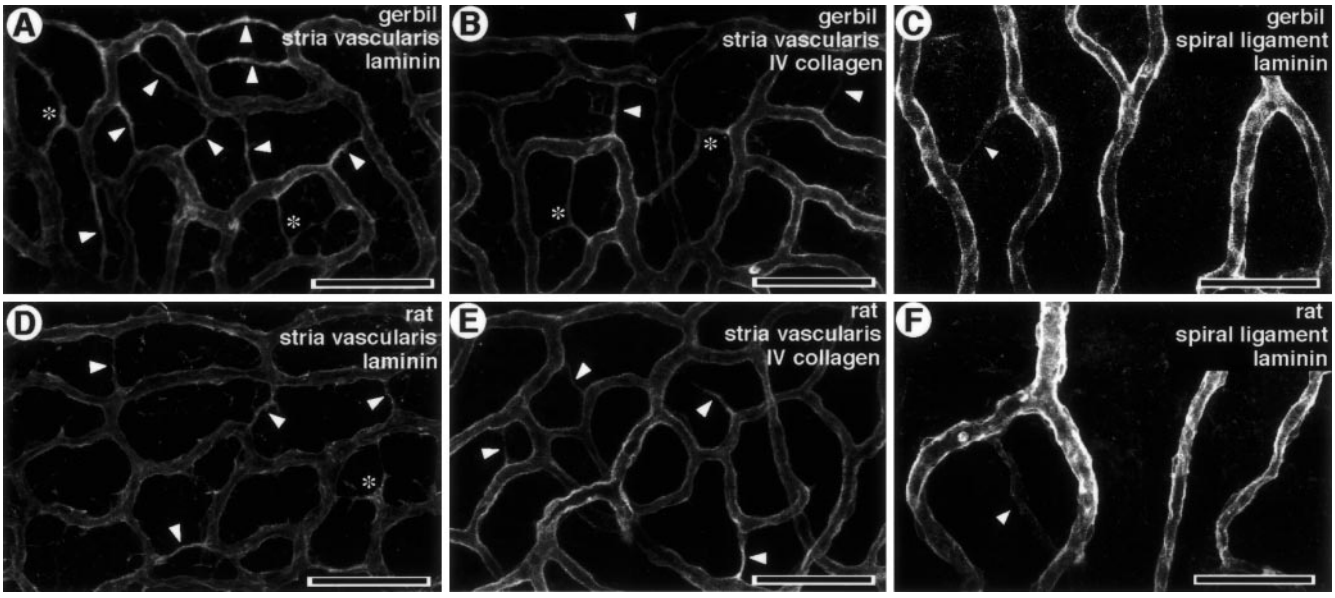
## Introduction

It is widely accepted that the stria vascularis in the cochlea plays a critical role in the generation of the positive endocochlear potential (EP) and the secretion of the endolymph, which contains a high concentration of $K^+$. Both the EP and the high $K^+$ concentration in the endolymph are essential for the normal sound transduction carried out by hair cells. The stria vascularis consists of marginal, intermediate, and basal cells and a dense capillary network. The capillary network in the stria vascularis is unique, because it lies between two cell layers connected by tight junctions; one is the epithelial marginal cell layer, and the other is the mesodermal basal cell layer. Numerous basal infoldings of the marginal cell and dendrite-like projections of the intermediate cell and the basal cell make close contact with the capillary. The capillary network in the stria vascularis is thought to be essential for sustaining the active metabolism required for the secretion of the endolymph and the production of the EP (Thalmann et al. 1972; Marcus et al. 1978). We have reported that both the capillary endothelial cell and the pericyte in the stria vascularis are dye-coupled with the intermediate cell and bridging structures between discrete capillaries have been identified (Takeuchi and Ando 1998). The purpose of this study has been to characterize morphologically the bridging structure between capillaries in the stria vascularis.

## Materials and methods

### Tissue preparation

Mongolian gerbils ($n$=16) and Wistar rats ($n$=13) were used. The protocol for the care and use of animals were approved by The Kochi Medical School Animal Care and Use Committee. All surgical treatments were performed under deep anesthesia (pentobarbital sodium: 50–100 mg/kg i.p.). Intravascular perfusion via the left ventricle was carried out with phosphate-buffered saline (PBS, pH 7.4), followed by a fixative. The cochleae were then perilymphatically perfused with the same fixative, and tissue strips (~ 2 mm long) of the stria vascularis with the underlying spiral




ligament were dissected and immersed in the fixative. The fixative for immunofluorescence preparations contained 4% paraformaldehyde and 0.1 M phosphate buffer (pH 7.4), and that for electron microscopy contained 2.5% glutaraldehyde, 2.0% paraformaldehyde, and 0.1 M phosphate buffer (pH 7.2).

**Fig. 1A–F** Immunologically visualized basal lamina in whole tissue preparations. Reconstructed projection images from serial optical sections taken at an interval of 1 µm. **A–C** Gerbil. **D–F** Rat. **A, B, D, E** Stria vascularis. **C, F** Spiral ligament. Laminin (**A, C, D, F**) or type-IV collagen (**B, E**) was immunostained (*arrowheads* bridging structures, *asterisks* bifurcated bridging structures). *Bars* 50 µm

### Labeling of basal lamina, filamentous actin, and capillary lumen

The basal lamina in whole tissue preparations was visualized immunologically. The fixed tissue strips were immersed in 3% sodium deoxycholate for 4 h at room temperature to facilitate the permeation of the antibodies (Hashimoto and Kusakabe 1997) and then pre-incubated in PBS containing 10% normal goat serum and 1% bovine serum albumin for 1 h. The specimens were subsequently exposed overnight at 4°C to polyclonal rabbit anti-rat laminin antibody (no. Z0097, DAKO, Glostrup, Denmark) or polyclonal rabbit anti-mouse type-IV collagen (no. LB1403, LSL, Tokyo, Japan) diluted 1: 200, and thereafter incubated for 6 h at room temperature in tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG (no. 111–025–003, Jackson ImmunoResearch Laboratories, West Grove, Pa., USA) diluted 1:100. In control experiments, the primary antibodies were substituted by pre-immune rabbit serum diluted 1:200, and no immunostaining was observed. Filamentous actin was visualized by exposing specimens to 0.17-µM Oregon-Green-conjugated phalloidin (Molecular Probes, Eugene, Ore., USA) for 6 h. The capillary lumen was visualized by using fluorescein isothiocyanate (FITC)-conjugated gelatin as reported previously (Ando and Takeuchi 1998).

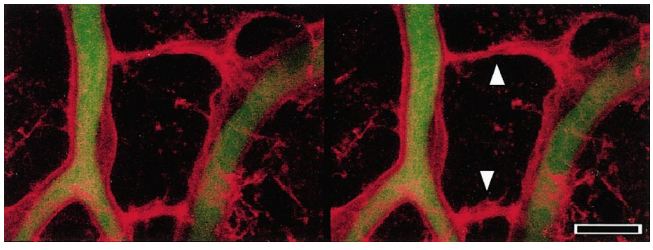


**Fig. 2** Double-labeling for laminin (*red*) and capillary lumen (*green*) in the gerbil stria vascularis. Reconstructed stereo-pair images. Note the absence of a capillary lumen in the bridging structures (*arrowheads*). *Bar* 10 µm

### Confocal laser microscopy and transmission electron microscopy

Observations of fluorescent images were made on a confocal laser scanning microscope system (LSM 410, Zeiss, Germany) equipped with a high magnification objective lens (100× or 63×). Fluorescence of TRITC was obtained by using a helium-neon laser (543 nm), a dichroic reflector (FT543), and an emission filter (FT570). Fluorescence of both Oregon Green and FITC was obtained by using an argon laser (488 nm), a dichroic reflector (FT510), and an emission filter (BP515–525). Tissue preparations for transmission electron microscopy were made as described previously (Ando and Takeuchi 1998) and examined on an electron microscope (H7100, Hitachi, Japan).

## Results and discussion

Both anti-laminin and anti-type-IV collagen antibodies reacted similarly with the tubular basal lamina around capillaries and permitted the visualization of the three-dimensional structure of the capillary network (Fig. 1). In addition to the capillary network, bridging structures between discrete capillaries were observed. These structures were distinguished from capillaries by their smaller diameter and the absence of a capillary lumen (Fig. 2). The immunofluorescence between the bridging structures and capillaries appeared to be continuous. Differences in the immunofluorescence patterns in gerbils and rats were not apparent (Fig. 1). A bifurcation of the structure was occasionally observed (Fig. 1), and branches of the structure also made contact with capillaries. Capillaries were often bent at the region where the bridging structures made contact (Fig. 1). The bridging struc-

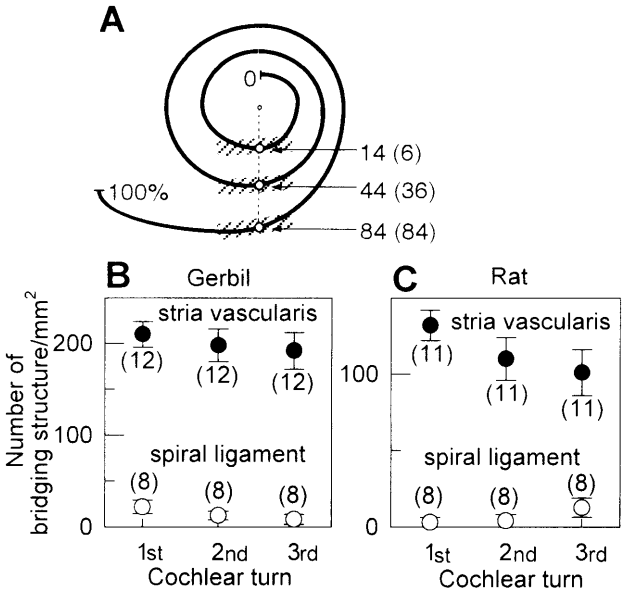



**Fig. 3A–C** Quantitative analysis of the bridging structure in the stria vascularis and the underlying spiral ligament. **A** Schema of the cochlea indicating approximate sampling positions (*shaded areas*). *Numbers* denote relative distances from the cochlear apex for gerbils and rats (in *parentheses*). **B, C** Density of the bridging structure in gerbils (**B**) and rats (**C**). *Closed* and *open symbols* The stria vascularis and the spiral ligament, respectively. Mean ± SEM. Number of observations in *parentheses*

**Fig. 4A–C** Electron micrographs of the gerbil stria vascularis. Sections were taken horizontal to the luminal surface of marginal cells. **A** Bridging cell between capillaries. The *rectangular area* is enlarged in **B**. *Arrowheads* Periphery of a bridging cell. **B** Border between a bridging cell and an endothelial cell. **C** Pericyte around a capillary. (*bl* Basal lamina, *cl* capillary lumen, *e* endothelial cell, *i* intermediate cell, *m* marginal cell, *p* pericyte.) *Bars* 5 μm (**A, C**), 0.5 μm (**B**)

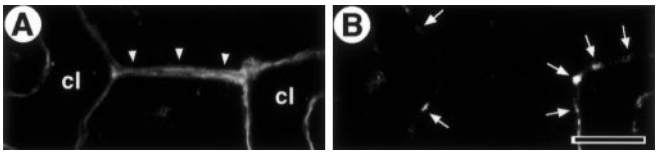
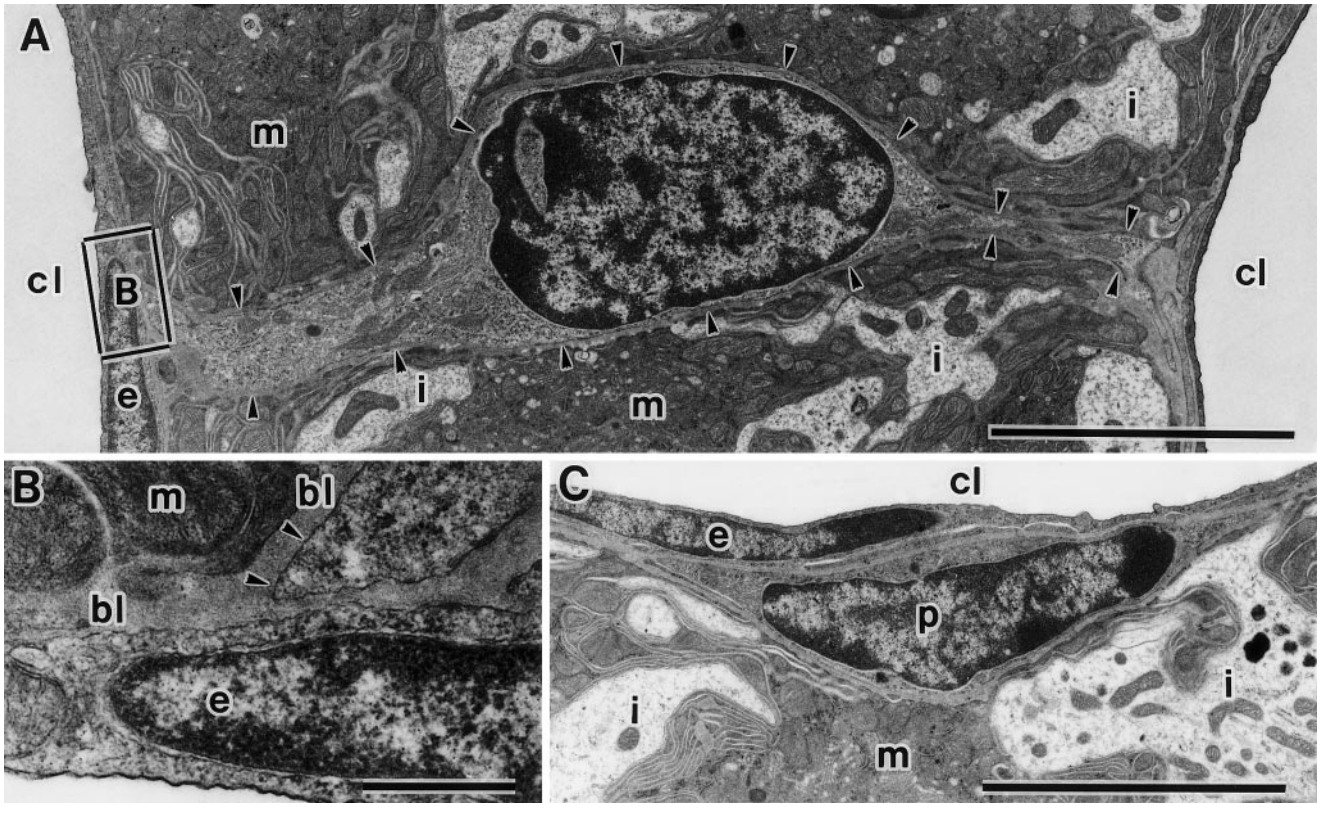



**Fig. 5A, B** Double staining for type-IV collagen (**A**) and filamentous actin (*arrows* in **B**) in the gerbil stria vascularis. Both images were obtained at the same plane for optical sectioning. (*Arrowheads* bridging cell, *cl* capillary lumen) *Bar* 10 μm

tures were also observed in the spiral ligament underlying the stria vascularis in both gerbils and rats (Fig. 1C, F). However, the density in the spiral ligament was much lower than that in the stria vascularis (Fig. 3).

To clarify the ultrastructure, we examined serial thin sections by electron microscopy (Fig. 4). The features of the bridging cells revealed by electron microscopy may be summarized as follows: (1) the bridging cell was surrounded by a basal lamina that was continuous with the basal lamina that enveloped the capillary endothelial cells, (2) only the ends of the bridging cell made contact with the endothelial cell, and (3) the electron density of the cytoplasm was similar to that of the pericyte and the endothelial cell (Fig. 4). A pericyte with usual morphology was also observed on the abluminal surface of the capillary endothelial cell (Fig. 4C). In view of the pericyte-like nature of the bridging cell, we examined the localization of filamentous actin in the bridging cell by using fluorescent dye-conjugated phalloidin, because pericytes around capillaries usually contain actin filaments. As shown in Fig. 5, filamentous actin was detected in pericytes around the capillaries, but not in the bridging cells.



Despite extensive immunohistochemical studies of the basal lamina in the stria vascularis (e.g., Takahashi and Hokunan 1992; Sagara et al. 1995; Sakaguchi et al. 1997), the bridging cell in the stria vascularis has not been reported until now. This may have been because of limited observations in cross-sections of the stria vascularis. We have been able to overcome this limitation by observing whole tissue preparations with the aid of a confocal laser microscope. Typically, the pericytes located around capillaries are defined by their topographical and morphological characteristics (Díaz-Flores et al. 1991), and various functions for pericytes have been proposed (Hirschi and D'Amore 1996). The bridging cell in the stria vascularis displays a number of similarities with pericytes, i.e., envelopment in a basal lamina, close contact with endothelial cells, and possession of a cytoplasmic electron density similar to that of pericytes, but differs in the paucity of its filamentous actin. Similar cells connecting discrete capillaries have been found in skeletal muscle (Williamson et al. 1980; Gaudio et al. 1990) and the skin (Imayama and Urabe 1984), but little is known about their functions. The glomerular mesangeal cell in the kidney may also bear morphological similarities to the bridging cell, including the attachment to capillaries and its relationship to the basal lamina (Kriz et al. 1995; Stockand and Sansom 1998).

In consideration of the proposed functions for pericytes and the anatomical and functional characteristics of the stria vascularis, we propose possible functions for the bridging cell. The first is that they may give mechanical strength to the capillary network and maintain its tortuous configuration. This would explain why the capillaries can often be seen to be bent at the site of bridging cell attachment. The tortuous capillaries in the stria vascularis probably result in a longer transit time for blood than that in straight capillaries; this would allow a more effective exchange of oxygen and metabolites. In contrast to the capillaries in most subepithelial tissues, capillaries in the stria vascularis do not have extracellular matrices, such as collagen fibers, except for the basal lamina around capillaries. As it is likely that the abundant blood flow within the strial vessels exerts a straightening force on the tortuous capillaries, mechanical reinforcement by the bridging cell may be needed to prevent the capillary network from being deformed. The difference in the density of the bridging structures between the stria vascularis and the spiral ligament (Fig. 3) supports the above proposal, as the capillaries in the spiral ligament are embedded in abundant extracellular matrices.

The second possibility relates to the intercellular communication in the stria vascularis. As the bridging cell is dye-coupled with the intermediate cell, basal cell, and the capillary endothelial cell (Takeuchi and Ando 1998), these coupled cells can exchange ions, metabolites, and messengers via the gap junctions. Thus, the bridging cell may play a role in the physiological function of the stria vascularis, i.e., the production of the EP and the endolymph. Although it has been generally accepted that pericytes around capillaries have filamentous actin (Skalli et al. 1989), and a contractile nature for pericytes has been reported (Tilton et al. 1979; Kelley et al. 1987), it seems unlikely that the bridging cell contracts like the common pericytes around capillaries, as filamentous actin in the bridging cell is not apparent.

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