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The presence of laminin in the fetal human inner ear

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Summary. The expression of laminin was analyzed in the human fetal inner ear using immunohistochemical methods. In the 11-week-old human fetus, the presence of laminin was found in the basement membrane of the immature cochlea, endolymphatic sac and vestibular end organs. The reaction of the basement membrane of the endolymphatic sac was strong in the 15-week-old human fetus. A laminin reaction was seen in the cochlea, Reissner's membrane, epithelial cells of the limbus spiralis, the basilar membrane and the stria vascularis. In particular, the capillaries and basement membrane of the stria vascularis were strongly positive. These results suggest that laminin may be an essential component in the development of the inner ear and may possibly be related to filtration of the endolymph.

Key words: Inner ear – Human fetus – Laminin

Introduction

The basement membrane is an extracellular structure containing both collagenous and non-collagenous glycoproteins [5]. Of these, laminin is a large non-collagenous glycoprotein and is one of the most important constituents of the basement membrane [13]. Laminin has been reported to bind to type 4 collagen and the glycosaminoglycan heparan sulfate to form a supramolecular complex [9]. It can also bind to the surface of epithelial and endothelial cells [12]. It has been suggested that laminin plays an important role in morphogenesis, especially in the genesis of the kidney [2]. A recent study by Zanetti et al. [14] has shown that a high frequency of natural antibodies against laminin was present in patients with sensorineural hearing loss. However, the laminin distribution in the human inner ear has not been described to date. As such, the present study investigated the content and distribution of laminin in the fetal human inner ear and has examined its functional role in the developing ear.

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Materials and methods

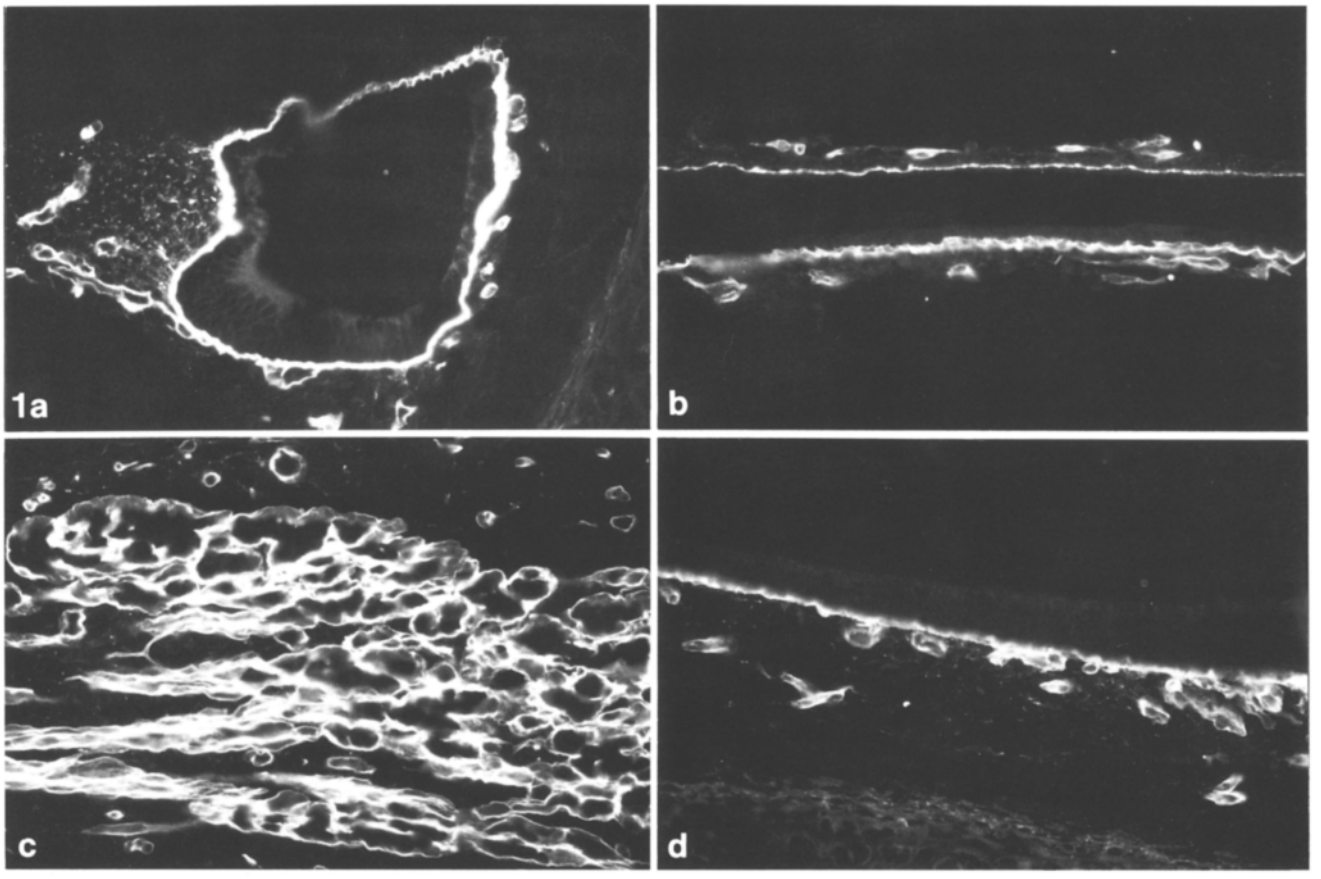
Inner ear specimens from the human fetus were used in this study. Two specimens were aged 11 weeks, two specimens were aged 14 weeks and four specimens were aged 15 weeks. All specimens were obtained after legal abortions. While the 11-week-old human embryos were obtained at surgery after curettage, the 14- and 15-week-old embryos were aborted following induction with prostaglandins. After the inner ears had been collected and dissected, they were immediately fixed in 4% paraformaldehyde in 0.01 *M* phosphate buffer saline (PBS) for 24 h. The specimens were then rinsed in PBS and embedded in paraffin after passing through a graded series of ethanols and 100% xylene.

The fetal inner ears in the paraffin blocks were sectioned serially in 5–6 μm thicknesses and mounted on glass slides with gelatin. Deparaffinized and hydrated sections were rinsed with PBS and treated with 0.1% pepsin (Sigma, St. Louis, Mo.) in 0.01 N HCl for 60 min at 37°C [3]. The treated sections were then incubated for 2 h at room temperature with a monoclonal antibody directed against human laminin. Antibody concentration was 5 μg/ml in PBS containing 1% bovine serum albumin (Boehringer, Mannheim, FRG). The sections were next washed with PBS and incubated with 2 μg/ml anti-mouse IgG-biotin (Boehringer) for 60 min at room temperature. After being washed with PBS, the sections were incubated with 20 μg/ml streptavidin fluorescein (Boehringer) for 60 min at room temperature. The sections were subsequently washed in PBS and mounted in glycerol. A Zeiss Axioplan fluorescence microscope (D-7082) was used for observation and photography. As controls, the same staining procedures were carried out in the absence of primary antibody or pepsin treatment.

Results

Without the pepsin treatment, no fluorescent reaction was found on any part of the tissue sections examined. In contrast, pepsin treatment for 60 min generally intensified the immunoreaction with laminin. The antigenicity of laminin was lost during the process of formalin fixation and paraffin embedding, but was restored by the 60-min pepsin treatment [3, 4].

In the 11-week-old fetus, the basement membrane of the immature cochlea was labelled with laminin. The basement membrane of the small blood vessels around the immature cochlea was also positive for laminin, but the subepithelial tissue of the cochlea was not labelled (Fig. 1a). Although the basement membrane of the en-



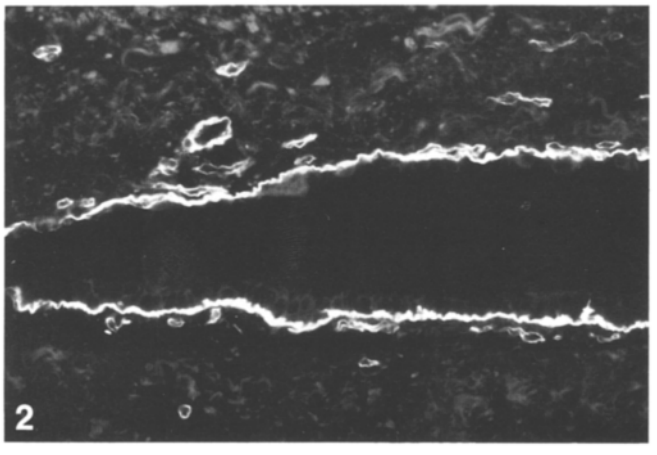


Fig. 1a–d. Detection of laminin in the 11-week-old human fetal inner ear. All micrographs were exposed for 30–40 s. × 400. **a** The basement membrane and capillary arteries of the immature cochlea are positive for laminin. **b** The basement membrane of the endolymphatic sac is laminin-positive. **c** The cell surface of the vestibular ganglion cells and nerve fibers is labelled by the antibody. **d** The basement membrane of the utricle is laminin-positive

Fig. 2. The 14-week-old human fetal endolymphatic sac. 20 s, × 400. The basement membrane and subepithelial tissue are labelled with laminin

dolymphatic sac and duct was positive, the subepithelial tissue was only weakly labelled while the epithelium itself was not stained at all (Fig. 1b). The lining membrane of the vestibular ganglion cells and nerve fibers was strongly positive (Fig. 1c). The basement membrane of the utricle was positive, as were the nerve sheath and blood vessels of the subepithelial tissue (Fig. 1d). The basement membrane of the crista ampullaris was weakly positive while the nerve sheath in the mesenchemal tissue was strongly positive.

In the 14-week-old fetus, the basement membrane of the endolymphatic sac was labelled with laminin. Labelling also occurred in the blood vessels and the connective tissue in the endolymphatic sac's subepithelial space (Fig. 2).

In the 15-week-old fetus, the reaction of the basement membrane and the connective tissue surrounding the endolymphatic sac was found to be strongly positive, but the epithelium was still laminin-negative (Fig. 3a). In the cochlea, Reissner's membrane, epithelial cells of the spiral limbus, the basilar membrane and the stria vascularis all reacted with laminin. The basement membrane and blood vessels of the stria vascularis in particular were strongly positive while the epithelial cells were negative (Fig. 3b, c). In the saccule, the basement membrane and nerve fibers in the subepithelial layer were positive while the epithelial cells were negative (Fig. 3d). In the vestibular ganglion, only the lining plasma membrane of the vestibular ganglion cells was strongly positive (Fig. 3e). These findings are summarized in Table 1.

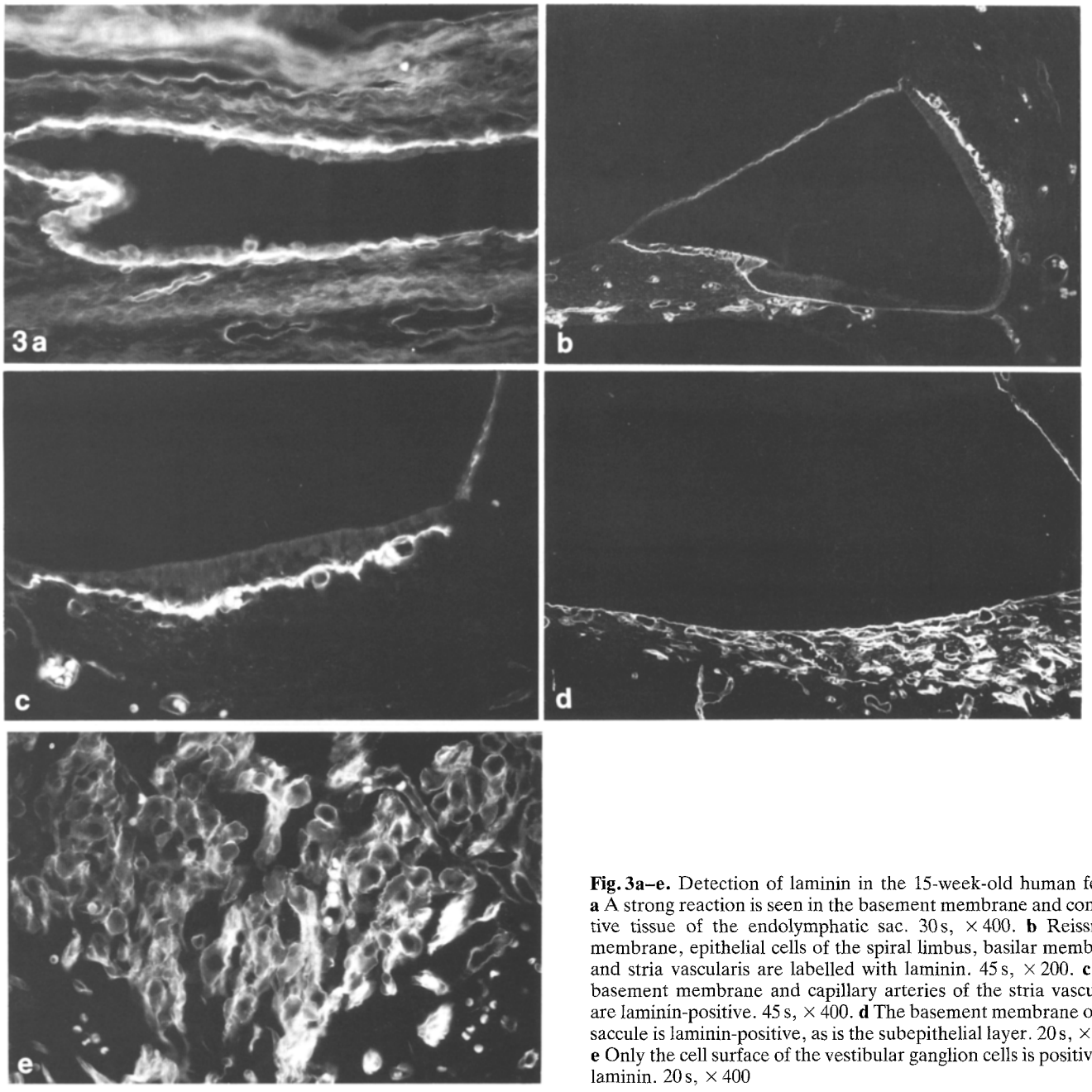


Fig. 3a–e. Detection of laminin in the 15-week-old human fetus. **a** A strong reaction is seen in the basement membrane and connective tissue of the endolymphatic sac. 30 s, × 400. **b** Reissner's membrane, epithelial cells of the spiral limbus, basilar membrane and stria vascularis are labelled with laminin. 45 s, × 200. **c** The basement membrane and capillary arteries of the stria vascularis are laminin-positive. 45 s, × 400. **d** The basement membrane of the saccule is laminin-positive, as is the subepithelial layer. 20 s, × 200. **e** Only the cell surface of the vestibular ganglion cells is positive for laminin. 20 s, × 400

Discussion

Laminin constitutes one of the major non-collagenous glycoproteins present in the basement membrane [9, 13] and has been considered responsible for many kinds of cell-to-basement membrane interactions, such as adhesion, migration or proliferation [9]. Laminin thus promotes the adhesion of many epithelial cells to type 4 collagen, including normal and tumorous cells [12] as well as certain other cells that are lined by a basement membrane, such as myoblasts and Schwann cells [10]. Furthermore, anti-laminin antibodies have recently been detected in certain acute and chronic infectious disorders. These include American cutaneous leishmaniasis [1], poststreptococcal glomerulonephritis [6] and Chaga's disease [11]. Anti-laminin antibodies have also been found in patients with sensorineural hearing loss [14].

The major functions of the basement membrane can be classified into two categories: firstly, it acts as a semipermeable filter and secondly, it acts as a supporting and/or a boundary structure [5]. As for a filtration function, the kidney glomerulus has been studied thoroughly. It is now known that the glomerulus performs not only mechanical filtration but also electrical filtration, based on negative charges in glycoproteins and glycosaminoglycan molecules. It has been suggested that laminin plays an important role as a constituent of the renal basement membrane for these functions [6]. It has also been inferred that laminin plays an important role in the morphogenesis of the kidney [2] and the lung [4].

Table 1. Expression of laminin in the inner ear of the 15-week-old human fetus

	Laminin
Inner ear ganglia	
Spiral ganglion	+ (cell membrane)
Vestibular ganglion	+ (cell membrane)
Köllikers organ	
Greater epithelial ridge	−
Lesser epithelial ridge	−
Tectorial membrane	−
Spiral limbus cells	+
Basilar membrane	+
Reissner's membrane	+
Stria vascularis	+ (blood vessels and basement membrane)
Subepithelial layer	+ (blood vessels)
Otolith organs	
Otoconia	−
Hair cells	−
Basement membrane	+
Subepithelial layer	+ (blood vessels and nerve fibres)
Crista ampullaris	
Cupula	−
Hair cells	−
Basement membrane	+ (dark cells)
Subepithelail layer	+ (blood vessels and nerve fibers)
Endolymphatic duct and sac	
Epithelial cells	−
Basement membrane	+
Subepithelial layer	+ (connective tissue and blood vessels)

There are strong indications that the endolymphatic sac and stria vascularis take part in the endolymphatic transport of water and ions [7, 8]. That the endolymphatic sac and stria vascularis are strongly positive for laminin, as shown in our present study, can be taken as an indication that laminin may be involved in the filtration of endolymph. Since a significant number of anti-laminin antibodies has been found in patients with sensorineural hearing loss, it is also possible that laminin may be involved in the pathogenesis of inner ear disease. One possible mechanism is its interaction with ion and fluid transport in the endolymphatic compartment.

The distribution of laminin in our present study was found to change as development of the embryos progressed. The significance of this is not known, but it is possible that lamin plays some role in the morphogenesis of the inner ear. While the basement membrane of the Schwann cells also contains laminin [10], we found that only the cell surfaces of the vestibular ganglion cells and nerve fibers were strongly positive for laminin. This may be an indication that laminin is part of the mechanism responsible for neural outgrowth.

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