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Laminins in the Adult and Aged Brain

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ABSTRACT

Only recently have we become aware of the diversity of laminins in adult brain. In vascular basement membranes, the expression of at least five laminin chains has been demonstrated, suggesting the presence of several laminin variants. Recent ultrastructural evidence for heterogeneity of laminin expression in vascular basement membranes is an exciting finding, and points to structural and functional diversity of the basement membranes around cerebral blood vessels. Neuronal laminin-like immunoreactivity in the adult brain is a consistent observation, but does not fit well in the current understanding of the physiology and biochemistry of heterotrimeric laminins. Nevertheless, the unique localization of putative neuronal laminins warrants their further characterization. The structure and function of laminins produced by reactive astrocytes in the lesioned adult brain and that seen in the brains of Alzheimer disease (AD) patients are not yet resolved. The possibility that these laminins play an important role in the CNS response to injury and pathophysiology of AD is expected to be a fruitful investigation. The next decade should see very significant advances in the characterization of brain laminins and, hopefully, in the elucidation of functional correlates to the structural diversity of laminins in brain.

Index Entries: Basement membrane; extracellular matrix; vasculature; blood vessel; astrocyte; endothelial cell; aging; Alzheimer disease; CNS; lesion, injury.

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INTRODUCTION

Laminins are known as a family of heterotrimeric large basement membrane (BM) proteins consisting of an α-chain, a β-chain, and a γ-chain (Timpl and Brown, 1994; Burgeson et al., 1994). Laminins mediate a variety of biological activities, and have been implicated in cell adhesion, cell migration, cell differentiation, and neurite outgrowth during brain development (Sanes, 1989). In the adult brain, the expression of most laminin chains is greatly reduced compared to developmental stages. Nevertheless, laminins are clearly detectable in the adult and aged brain, but their nature and function are less defined.

LAMININS ARE COMPONENTS OF BASEMENT MEMBRANES IN ADULT BRAIN

BM in brain is restricted to blood vessels, pia mater, ependyma, and choroid plexus. Laminins appear to be consistent components of all cerebral BMs. In BM of blood vessels, immunohistochemistry with laminin chain-specific antibodies suggests the presence of the laminin α1, α2, β1, β2, and γ1 chains (Hagg et al., 1989; Hunter et al., 1992; Jucker et al., 1996; *see also* Fig. 1). Specificity of the staining for α2 has been demonstrated in an α2 mutant mouse, which lacks α2 immunoreactivity of blood vessel BM (Jucker et al., 1996). The presence of the β1 and γ1 chain in vascular BM is supported by *in situ* hybridization in the embryonic brain revealing β1 and γ1 transcripts associated with differentiating blood vessels (Fig. 1C; Thomas and Dziadek, 1993). In adult brain, transcripts for laminin chains are greatly reduced and do not easily allow their localization *in situ* (unpublished results), an observation suggesting long half-lives of laminins in BM.

The presence of α1, α2, β1, β2, and γ1 in blood vessel BM suggests that laminin-1, laminin-2, laminin-3, and laminin-4 (for nomenclature, *see* Burgeson et al., 1994) might all be components of the laminin network within the cerebral vascular BM. Although it cannot be ruled out that laminin variants other than heterotrimeric ones exist, no evidence has been presented so far. Moreover, immunoblots of BM laminin extracts from brain electrophoresed under nonreducing conditions and probed with α2 and γ1-specific antibodies, as well as with antibodies to laminin-1, revealed bands at 700–900 kDa, suggesting the presence of trimeric molecules (Hagg et al., 1989; H. Hall, London, unpublished results). The presence of multiple laminins within the vascular BM in brain appears to be in contrast to most peripheral BMs, where laminins have an almost exlusive distribution (Sanes et al., 1990; Engvall et al., 1990). Recent studies in our laboratory, however, suggests that BM heterogeneity might in fact also occur within BMs around cerebral blood vessels. At the ultrastructural level, preliminary results indicate that the γ1 chain is a component of

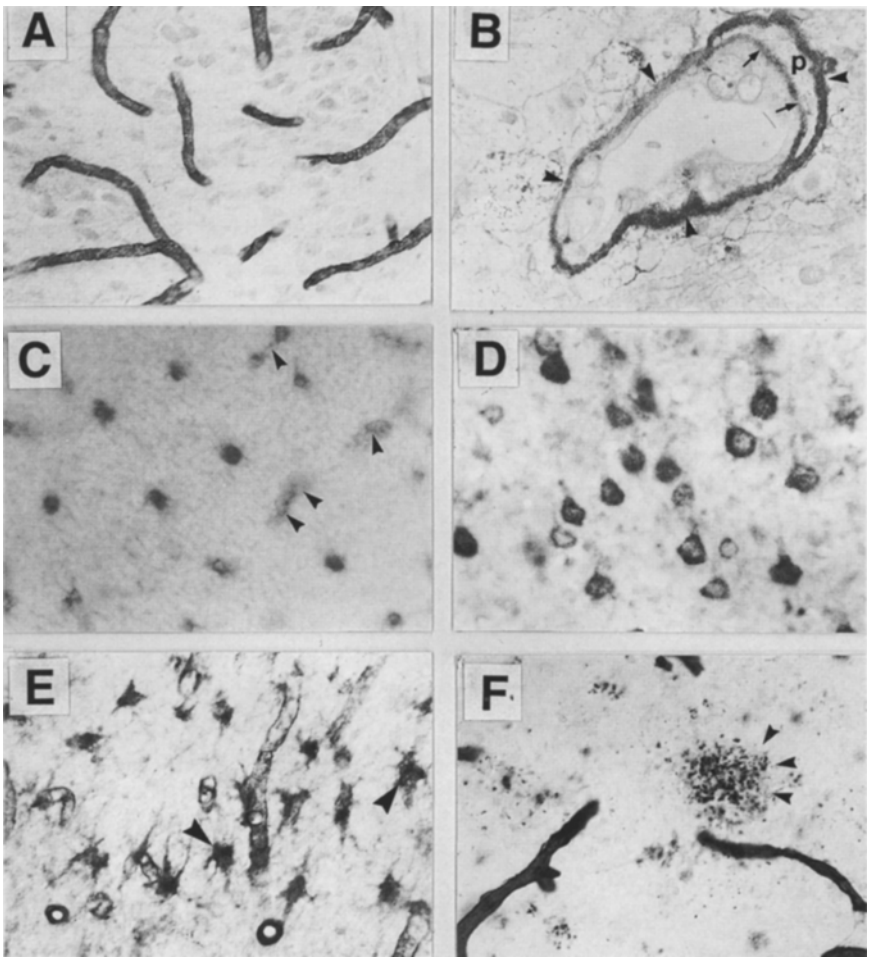

Fig. 1. Laminin-like immunoreactivities in the mammalian brain. (**A**) Monoclonal antibodies to the laminin γ1 chain (2E8; Engvall et al., 1990) immunolabels blood vessel throughout brain. Shown here is the rat entorhinal cortex, which was only moderately fixed prior to immunostaining. (**B**) At the electron microscopic level, γ1 labeling is associated with the endothelial BM (arrows) and the parenchymal/glial BM (arrowheads). p = *pericyte.* (**C**) *In situ* hybridization with γ1-specific digoxygenin-labeled cRNA probes localizes transcripts to developing capillaries in the E13 mouse brain (arrowheads). (**D**) Widespread intraneuronal labeling is observed in well-fixed normal adult brain with γ1-specific antibody. Shown here is the rat parietal cortex. Blood vessel labeling is absent, since thorough formaldehyde-fixation masks vascular BM antigenicity (Jucker et al., 1992a). (**E**) In response to brain injury, astrocytes become laminin-immunoreactive (arrowheads). Shown here is the rat hippocampus 16 d after transient ischemia and labeled with γ1-specific antibody. (**F**) In brain tissue from a patient with AD, punctate accumulations of laminin-like immunoreactive material (here labeled with γ1-specific antibody 2E8) is often associated with senile plaques (arrowheads).

both the BM overlying the endothelial cells (endothelial BM) and the BM associated with perivascular astrocytic end feet (parenchymal or glial BM) (Fig. 1B), whereas α2 appears only to be a component of the parenchymal/glial BM (Jucker et al., 1996). Often endothelial BM and parenchymal BM fuse to form one single perivascular BM, but two BMs can easily be distinguished around pericytes (Peters et al., 1991). BM in the normal adult brain is thought to mediate primarily a supportive, containment, and barrier function. Cerebral BM heterogeneity might serve selective barrier functions in normal adult brain and play an important role in repair after brain injury.

Recent evidence suggests that BMs in brain are assembled from components secreted in a coordinated fashion from different cell types (Thomas and Dziadek, 1993). Assuming that laminin subunits are produced and deposited by cells in close contact with BM, vascular endothelial cells, pericytes, and perivascular astrocytes (and smooth muscle cells for the BM around arteriols and arteries) are the likely sources of vascular BM laminin. Similarly, pial astrocytes, leptomeningeal cells, and choroidal epithelial cells are likely the laminin-secreting cells for the BMs associated with the pia and choroid plexus. In culture, immature astrocytes produce α2, β2 and γ1, but not α1 and β1 (Liesi and Risteli, 1989; Wujek et al., 1990; Chiu et al., 1991; Morisette and Carbonetto, 1995). Cerebral vascular endothelial cells and leptomeningeal cells have also been reported to produce laminin in culture, but the chain composition has not been examined (Rutka et al., 1986; Pákáski et al., 1990). Results from in vitro studies should be interpreted with caution, since they do not reflect accurately the complex in vivo assembly of BM. Moreover, cells in culture might produce laminin subunits not observed in vivo, and the pattern of laminin chain expression could change with maturation (e.g., Hunter et al., 1992). Nevertheless, based on the above-mentioned cell-culture results and the recent immunoelectron microscopic localization studies, our hypothesis is that perivascular astrocytes secrete α2, β2, and γ1 (laminin-4), whereas vascular endothelial cells secrete α1, β1, and γ1 (laminin-1). Where parenchymal/glial BM and endothelial BM fuse, all five laminin chains appear to be present.

IS THERE A NEURONAL LAMININ?

Laminin-like immunoreactivity has been reported in neurons and their processes using polyclonal antibodies to laminin-1, and monoclonal chain-specific antibodies to α2 and γ1 (Yamamoto et al., 1988; Hagg et al., 1989, 1996; Zhou, 1990; Tian et al., 1994; Morisette and Carbonetto, 1995; *see also* Fig. 1D). This neuronal laminin immunoreactivity appears somewhat dependent on tissue preparation and staining technique (Fig. 1A vs D) (Hagg et al., 1989; Jucker et al., 1992a). *In situ* hybridization with β1-specific probes indicated synthesis of β1 mRNA by retinal ganglion cells

(Sarthy and Fu, 1990), and *in situ* hybridization with β1- and γ1-specific probes revealed labeling of peripheral dorsal root ganglion neurons LeBeau et al., 1994). Although neuronal laminin-like immunoreactivity in adult brain appears to be a consistent observation, caution in the interpretation is warranted. No convincing laminin expression or secretion has yet been observed in cultured neurons, and *in situ* hydridization with laminin chain-specific probes has not yet revealed neuronal labeling corresponding to the widespread neuronal immunoreactivity in brain. Hagg et al. (1989) suggested that glial or nonneuronal cells in the brain might produce laminin and supply it to neurons. Given the growing family of laminin variants, it is also conceivable that neuronal laminins represent new laminin variants that share immunological crossreactivity with other laminin members.

Recently, we have initiated studies to characterize the laminin α2-like neuronal immunoreactivity in brain in more detail (Hagg et al., 1996; Tian et al., 1994). At the ultrastructural level, immunoreactivity in the hippocampus is associated with dendrites and dendritic spines, and a role in synaptic function of this laminin α2-like antigen has been suggested (Tian et al., 1994). Although immunoblots from adult hippocampus, hippocampal synaptosomal fraction, and primary hippocampal cell culture provide some evidence for genuine α2 expression in neurons, other results suggest an α2 isoform that is processed very similarly to the laminin α2 chain (Tian et al., 1994).

LAMININ EXPRESSION IN RESPONSE TO BRAIN INJURY

The normal adult brain reacts to a penetrating (mechanical) injury with the formation of a new glia limitans and laminin-positive BM, which divides the scar tissue from the underlying neuropil (Bernstein et al., 1985; Suzuki and Choi, 1990). Moreover, an increase in number, intensity, and size of laminin-positive blood vessels in and around the lesion site has been observed (e.g., Eriksdotter-Nilsson et al., 1987; Giftochristos and David, 1988; Hagg et al., 1989). However, although the increase in number of laminin-positive vascular elements could correspond to re- and neovascularization after injury, the increase in staining intensity might partly be attributed to an unmasking of laminin epitopes in pre-existing vasculature and/or to differential penetration of antibodies to the vascular BM of intact vs lesioned tissue (Krum et al., 1991; Jucker et al., 1992a).

Laminin-positive astrocytes are generally absent in normal adult mammalian brain. In contrast, after CNS injury, laminin-positive reactive astrocytes appear around the lesion site. This observation has been made with polyclonal antibody to laminin-1, and monoclonal antibody to the γ1 chain after mechanical (e.g., Bernstein et al., 1985; Giftochristos and David, 1988; Hagg et al., 1989) as well as ischemic and neurotoxic lesions (Liesi et al., 1984; Jucker et al., 1993). Laminin immunoreactivity is confined to the astrocytic cell body and proximal processes (Fig. 1E). Other

studies failed to observe laminin immunoreactivity associated with reactive astrocytes (e.g., Stichel and Muller, 1994), suggesting the importance of the type of injury and brain region, and/or methodology. Astrocyte-derived laminin after injury may be secreted and become incorporated into BM of newly formed vessels. Alternatively, astrocyte-derived laminin might be deposited on the cell surface or secreted into the extracellular space of the lesioned brain.

Laminin secreted by cultured immature astrocytes (*see above*) is not necessarily the same as the laminin secreted by reactive astrocytes after brain injury. During development, glial-derived laminin guides and promotes axon growth in certain brain regions (Liesi and Silver, 1988; Gordon-Weeks et al., 1989; Zhou, 1990). In contrast, in the lesioned adult brain, the distribution of laminin-positive astrocytes appears not to coincide with axonal sprouting (Giftochristos and David, 1988; Stichel and Muller, 1994). Thus, astrocyte-derived laminin in the lesioned brain might have entirely different functional and structural properties than glial-derived laminin in the developing brain. Although most laminins are clearly neurite outgrowth promoting for CNS neurons, it is conceivable that some laminin variants have an inhibitory role on regenerating CNS fibers (e.g., Stichel and Muller, 1994).

DEPOSITS OF LAMININ IMMUNOREACTIVE MATERIAL IN THE AGED BRAIN

In brains of Alzheimer disease (AD) patients, punctate laminin-immunoreactive material colocalizes with senile plaques (Perlmutter and Chui, 1990; Murtomäki et al., 1992). Besides its immunolabeling with polyclonal antibodies to laminin-1 and monoclonal antibodies to the γ1-chain (Fig. 1F), the composition of this laminin-like material has not been studied in more detail. It has been proposed that this plaque-associated laminin-like material represents vascular BM laminin from degenerated capillaries, or might be produced and deposited by glial cells (Perlmutter and Chui, 1990). Coarse laminin-immunoreactive material is also observed around amyloid-laden vessels in patients with hereditary cerebral hemorrhage with amyloidosis Dutch-type, and its localization suggests a vascular origin (van Duinen et al., 1995). Laminin β1- and γ1-chain transcripts appear elevated in AD brain compared to age-matched controls with γ1 expressed at a significantly higher level than β1 (Murtomäki et al., 1992). Weak laminin immunolabeling of glial cells has been observed in AD, but no *in situ* localization of laminin transcripts has yet been reported. Other vascular BM components, such as heparan sulfate proteoglycan (HSPG), are also consistent components of AD plaques (Snow and Wight, 1989). Laminin-1 and HSPG bind the β-amyloid precursor protein, which is central to the pathophysiology of AD, and it has been suggested that the deposition of BM components is an important initial event in plaque for-

mation in AD (Perlmutter and Chui, 1990; Narindrasorasak et al., 1995). Most laminin variants are neurite outgrowth-promoting, and the deposition of laminin in plaques or its interaction with the β-amyloid precursor protein might mediate the characteristic aberrant sprouting response in AD brain (Kibbey et al., 1993). However, to move beyond speculations about function, it is necessary to characterize this plaque-associated laminin-like material beyond its immunochemical characteristics.

Recently, age-related deposits of aggregated fibrillar material have been described in brains of certain mouse strains (Jucker et al., 1992b; 1994). The deposits are associated with astrocytic processes and appear to contain laminins and HSPG. The deposits do not represent senile plaques with β-amyloid deposition, but they may mimic the deposition of laminin and HSPG in AD plaques. Further characterization of this laminin-like and HSPG-like material in aged murine brain might reveal insights into the origin and nature of laminin and HSPG in AD brain.

CONCLUSION

Given their many biological activities, laminins in adult, aged, and lesioned brain are of considerable interest. The majority of studies on laminins in brain have been conducted before the existence of multiple laminin variants was recognized. Most of these studies have used antibodies against EHS laminin (now laminin-1; Burgeson et al., 1994) with undefined crossreactivity to the later-discovered members of the laminin family. Today, ten genetically different laminin chains are known and strikingly, most of them appear to be expressed in brain (for α1, α2, β1, β2, γ1, *see above;* for the more recently cloned α3, α5, and γ2, *see* Kallunki et al., 1992; Galliano et al., 1995; Miner et al., 1995). Thus, further characterization of brain laminins appears necessary and promising in elucidating functional correlates to the structural diversity of laminins in brain.

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