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Vimentin immunoreactivity in normal and pathological human brain tissue*

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Summary. Vimentin immunoreactivity was examined in brain tissues from non-neurological and various human central nervous system disease cases. In all brain tissues examined, vimentin immunoreactivity was intensely positive in ependymal cells and subpial tissues, and weakly positive in some capillaries and some white matter astrocytes. In affected areas of Alzheimer's disease (AD), Pick's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and cerebral infarction cases, numerous intensely vimentin-immunopositive astrocytes of both protoplasmic and fibrous morphology were demonstrated. A few such astrocytes were also observed in Parkinson's disease and progressive supranuclear palsy. ALS, MS and infarction brains also had numerous, strongly vimentin-positive, round and fat-laden microglia/macrophages. In AD and ALS, a few reactive microglia with irregularly enlarged shapes were vimentin positive. In AD, they were almost exclusively related to senile plaques.

Key words: Vimentin – Astrocyte – Microglia – Macrophage – Alzheimer's disease

Vimentin is the major protein in one type of intermediate filament. Intermediate filaments themselves are broadly distributed in eukaryotic cells. They have a characteristic diameter of 7–11 nm, in between those of actin filaments (5–7 nm) and microtubulin polymers (22–25 nm). They form a distinctive fibrous cellular network. There are five intermediate filament families, with vimentin being a product of a type III gene [15]. The others are glial fibrillary acidic protein (GFAP), neurofilament, desmin, and keratin. A particular intermediate filament family will often be characteristic of a cell type, but different intermediate filaments may coexist in the same cells. For example, vimentin and GFAP often coexist in astrocytes [4, 14, 16, 17, 19, 20, 23] and, in gliomal cell lines, can even be codistributed in the same intermediate filament system [17]. Although the functions of intermediate filaments are unknown, they have attracted the attention of pathologists because of their value in tracing neoplastic cell lineages [15, 22]. Vimentin containing fibers are seen in cells of mesenchymal origin [3, 6, 15, 22] as well as various epithelial cells and cells of neoplastic origin [15, 22]. In the central nervous system (CNS), immature [4, 16, 19, 21, 23], reactive [18, 20, 24] and neoplastic astrocytes [17, 22] express this protein. Ependymal [21], endothelial [5, 6] and microglial [6, 7] cells have also been reported to express vimentin.

Vimentin has many structural similarities to GFAP. It is, therefore, of potential interest in neuropathology. In the adult human CNS, only astrocytes in normal cerebral cortical smears [1] and cerebellar tissue from Creutzfeldt-Jakob disease [10] have been reported as vimentin positive. We present in this report a more comprehensive survey of vimentin-immunoreactive structures in brain tissues from various human CNS disorders, as well as cases free of neurological disorder. In normal tissue, we find that vimentin is moderately to strongly expressed in ependymal, choroid plexus and meningeal cells. It is also moderately to strongly expressed in some subpial astrocytes and weakly expressed in endothelial cells and a few white matter astrocytes. In pathological tissue, it is very strongly expressed in some hypertrophied astrocytes, and some lipid-laden reactive microglia/macrophages.

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Materials and methods

The materials we used came from ten cases of Alzheimer's disease (AD) (age 56–81 years), four cases of amyotrophic lateral sclerosis (ALS) (age 48–81 years), two cases of Pick's disease (ages 72, 87 years), three cases of multiple sclerosis (MS) (age 56–66 years),

three cases of cerebral infarction (age 74–87 years), six cases of Parkinson's disease (PD) (age 61–82 years), three cases of progressive supranuclear palsy (PSP) (age 60–66) and six cases free of neurological disease (age 54–82 years).

Brains in all cases were obtained 2–32 h after death. Small blocks of tissue were dissected from the angular and entorhinal cortices and hippocampus of AD brains, the precentral cortex and the cervical spinal cord of ALS brains, frontal and temporal cortices of Pick's disease brains, plaque areas of MS brains, infarcted areas of stroke brains, the substantia nigra of PD brains, the striatum and globus pallidus of PSP brains and various brain regions of non-neurological cases. Blocks were fixed for 2 days in phosphate-buffered 4 % paraformaldehyde and then transferred to a maintenance solution of 15 % sucrose in 0.1 M phosphate buffer, pH 7.4, in which they were kept, in the cold, for periods up to a few weeks until used. Sections cut from blocks maintained under such conditions for over 4 years show no deterioration in immunohistochemical characteristics. Sections were cut on a freezing microtome at 30 μm thickness, washed and stained by single- or double-immunohistochemical procedures as previously described [9, 12, 13]. The antibodies and dilutions used were: anti-vimentin, 1:1000 (mouse monoclonal, Sanbio); anti-leukocyte common antigen (LCA), 1:100 (mouse monoclonal, Dako); anti-HLA-DR, 1:1000 (mouse monoclonal, American Type Culture Collection); anti-glial fibrillary acidic protein (GFAP), 1:10000 (rabbit polyclonal, Dako); and anti-β-amyloid protein (BAP, rabbit polyclonal antibody to synthetic peptide 1–24 of BAP, 1:10,000) [8]. Some sections were counterstained with Oil red O as previously described [2].

For electron microscopy, blocks of cortical areas from three cases of AD were fixed in 1 % glutaraldehyde/4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 h at 4 °C, followed by immersion in 15 % sucrose in 0.1 M phosphate buffer, pH 7.4, for several days at the same temperature. Sections were cut by vibratome at 50 μm thickness and incubated with anti-vimentin antibody (1:1000) for 5 days at 4 °C. They were then treated with the appropriate Vectastain-avidin-biotin-peroxidase complex second antibody systems. After the diaminobenzidine reaction, the sections were osmified, dehydrated and embedded in Epon. Ultrathin sections were cut and examined without counterstaining with a Philips EM201 electron microscope.

The specificity of the anti-vimentin antibody was tested by immunoblot analysis using AD brain tissue. A sample of temporal cortex gray matter was homogenized in buffer (TRIS-HCl, pH 7.4, 1 mM EGTA, 1 mM EDTA, 0.1 mM leupeptin, 0.1 mM phenylmethylsulfonyl fluoride). A sample of the homogenate was dissolved in 2 × SDS sample buffer (without reducing agent), and 50 μg samples of this were separated on a 9 % SDS-polyacrylamide gel. The separated proteins were electroblotted to a nitrocellulose membrane. The membrane was pretreated with 5 % skim milk powder in TRIS-buffered saline (TBS) and then incubated with anti-vimentin antibody (1:1000) in 2 % skim milk/TBS, for 18 h at 4 °C. The membrane was extensively washed in TBS + 0.05 % Tween 20, and the bound antibody labelled with alkaline phosphatase-conjugated anti-mouse antibody (BRL, 1:5000, 2 h at room temperature). Following further washing, the membrane was developed in alkaline phosphatase substrate buffer [0.33 mg/ml nitroblue tetrazolium (NBT), 0.44 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in 0.1 M TRIS-HCl (pH 9.5) plus 50 mM NaCl, 50 mM $MgCl_2$].

Results

A single band with molecular mass of approximately 55 kDa was detected in an AD brain sample by Western blotting, confirming the specificity of the antibody (Fig. 1).

The only structures that always stained for vimentin were those lining the surfaces of the brain. Choroid plexus cells (Fig. 2A), ependymal cells (Fig. 2B), and the pial membrane, as well as some glial cells near the pial surface (Fig. 2C), were moderately to intensely positive in all cases examined. In non-neurological cases, some capillaries and endothelial cells associated with larger vessels were weakly stained (Fig. 2D). In addition, a few normal-appearing astrocytes in white matter were weakly positive (Fig. 2E). Their numbers were small compared with the numbers of GFAP-positive astrocytes (Table 1). No positive staining was obtained when the primary antibody was omitted or a monoclonal antibody indifferent to brain tissue substituted (Fig. 2F).

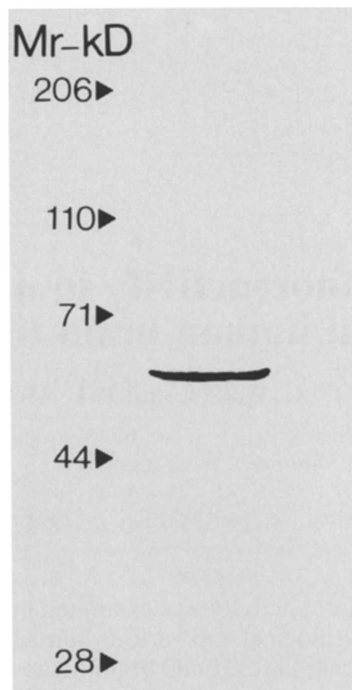

Fig. 1. Immunoblot from the Alzheimer's disease brain sample. A single band with molecular mass of approximately 55 kDa is seen. See Materials and methods for details

In pathological tissue from all diseases examined, including cerebral infarction cases, some strongly vimentin-positive, hypertrophied astrocytes of both protoplasmic (Fig. 3A) and fibrous (Fig. 3B) morphology were observed (Table 1). Astrocytes of protoplasmic morphology outnumbered those of fibrous morphology but, particularly in MS, fibrous forms were dominant within plaque areas (Fig. 3B). In AD, vimentin-positive fibrous astrocytes were located at the margins of some senile plaques (Fig. 3C). The AD senile plaque areas were outlined by double immunostaining with anti-BAP. Vimentin-positive astrocytic fibers often penetrated the plaques (Fig. 3C). Double immunostaining with antibodies to vimentin and GFAP established coexistence of these two intermediate filament proteins in many of the hypertrophied astrocytes (data not shown).

Vimentin-positive, round and fat-laden microglia/macrophages were demonstrated in the lateral and anterior columns of the spinal cord and in the white

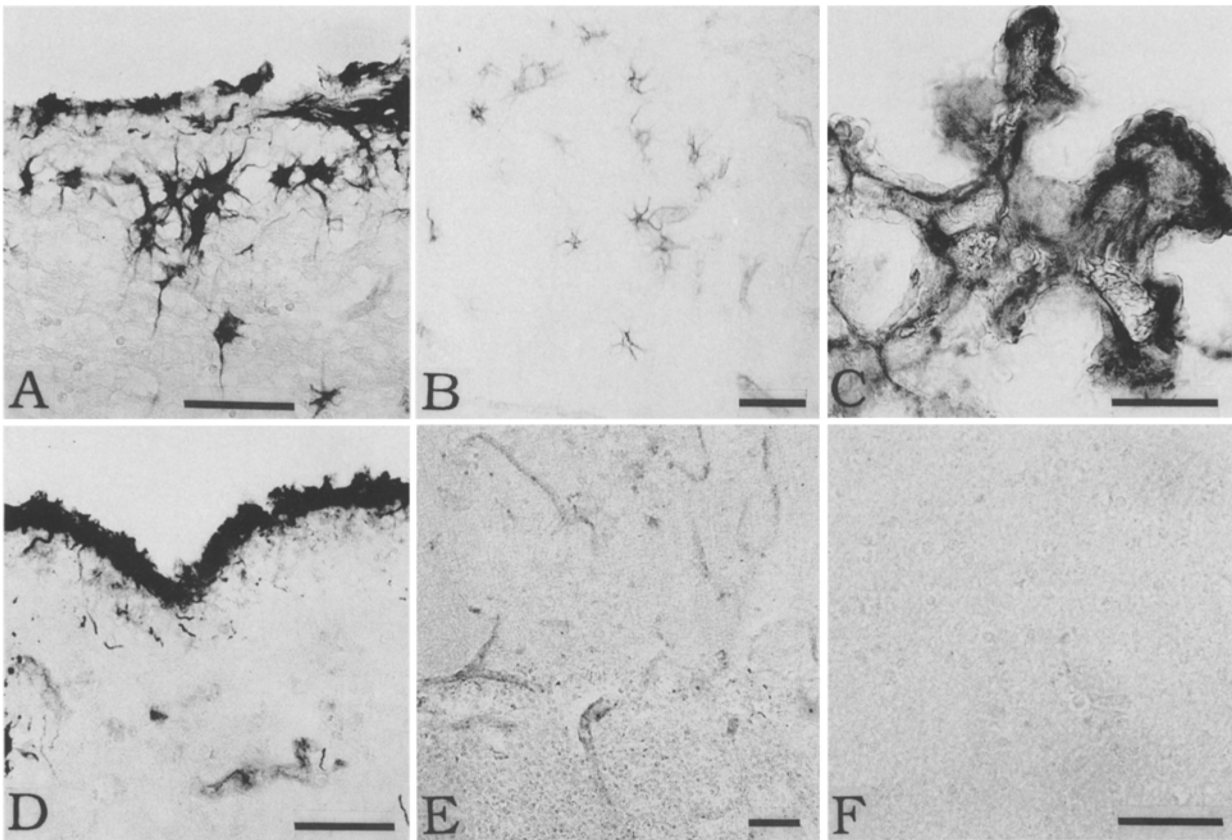

Fig. 2A–F. Immunohistochemistry of vimentin in control brains. **A** Superficial glial limiting membrane and subpial astrocytes showing vimentin immunoreactivity in frontal cortex of a normal control. **B** Vimentin-positive normal appearing astrocytes as well as capillaries (endothelial cells) in control brain tissue. **C** Choroid plexus tissue positive for vimentin in normal control brain. **D** Ependymal cell layer and some processes of subependymal astrocytes heavily labelled for vimentin at the lateral ventricle margin of the caudate in a normal control brain. **E** Cerebellar cortex from a normal control showing only weak staining of capillaries for vimentin. **F** Frontal cortex stained without the primary antibody. *Bars* = 50 μm

matter of the precentral gyrus in ALS (Fig. 3D), at the margin of the plaques in MS cases, and in infarcted areas of stroke cases. Counterstaining with Oil red O produced an intense cherry red color in these lipid-laden, vimentin-positive cells, indicative of myelin breakdown products [2]. Vimentin-positive cells, with hypertrophied cytoplasm and thickened processes typical of reactive microglia, were also occasionally seen in association with AD senile plaques (Fig. 3E). Again, the outline of the plaques was demonstrated by double immunostaining with anti-BAP.

Double immunostaining was also carried out with anti-vimentin in combination with either anti-LCA or anti-HLA-DR. LCA is expressed by all microglia [9], while HLA-DR is expressed by reactive microglia [9, 12, 13]. Such double immunostaining revealed that only a small proportion of the LCA-positive (Fig. 3F) and HLA-DR-positive microglia were also vimentin positive. In gray and white matter in the precentral gyrus of ALS cases, where reactive microglia were seen by staining with anti-HLA-DR antibody, a few vimentin-positive microglia were also seen.

Ultrastructural studies of AD brains revealed that vimentin immunoreactivity occurred in reactive astrocytes (Fig. 3G, H), reactive microglia (Fig. 3I, J) and endothelial cells (Fig. 3K, L). Vimentin-positive microglial cells and their processes were located near the core of AD senile plaques. They had amyloid fibers in the cytoplasm, which communicated with the amyloid star [25]. Vimentin-positive astroglial processes also projected to the amyloid core or star of AD plaques (data not shown).

The overall results showing the relative frequency of vimentin-positive structures, as well as the intensity of staining, is shown in Table 1.

Discussion

The distribution of vimentin-positive structures in normal human brain tissue generally paralleled that reported by Mares et al. [11] for adult rat, except that Bergmann glia were not positive in adult human cerebellum. Some astrocytes and endothelial cells were weakly positive, while ependymal cells, choroid plexus cells, meningeal layers and supendymal fibrous layers were more strongly stained.

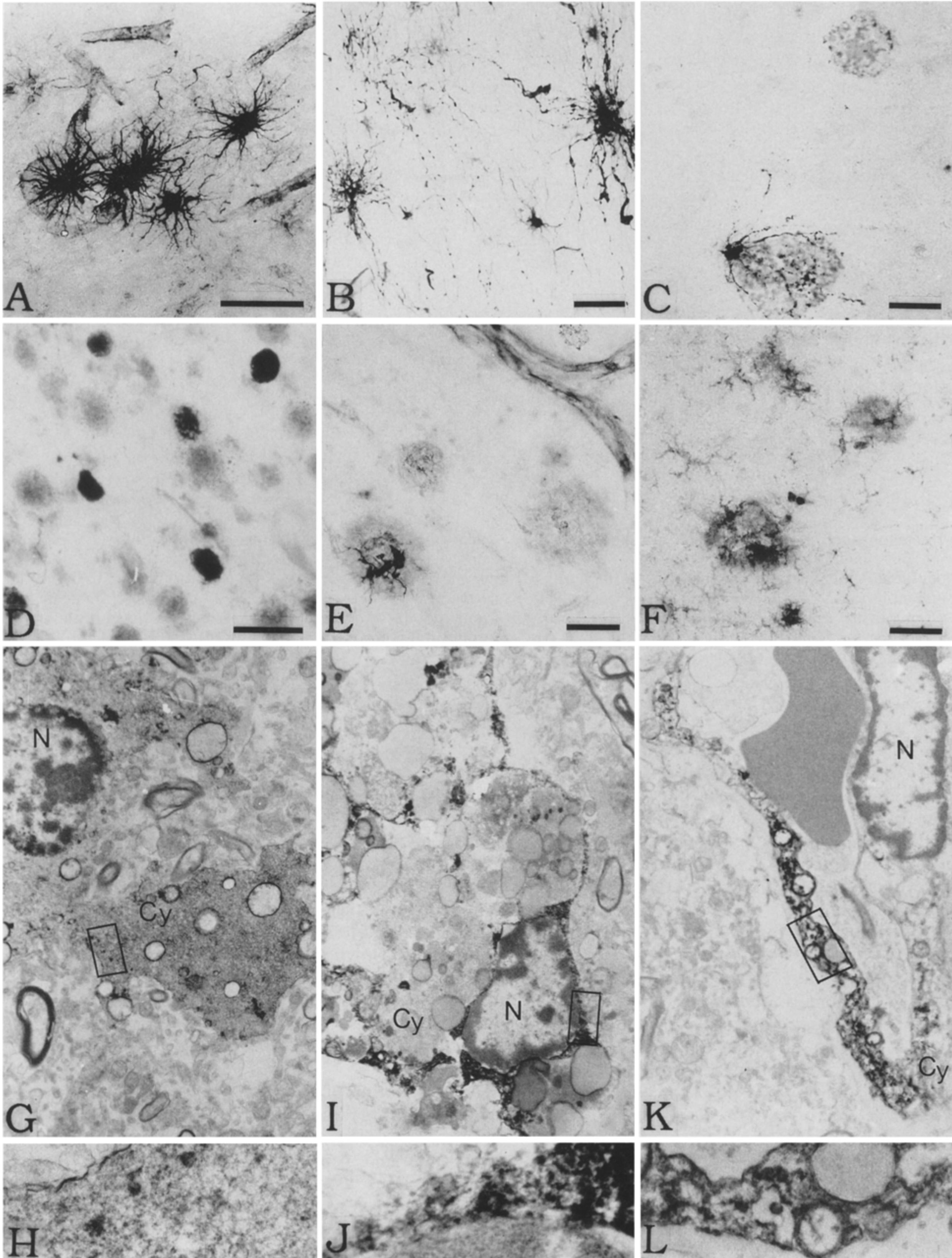
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Table 1. Intensity and frequency of vimentin-positive structures in normal and neurologically diseased brains[a]

	Normal		Neurologically diseased	
	Intensity	Frequency	Intensity	Frequency
Ependymal cells	3–4	++++	3–4	++++
Choroid plexus	2–3	++++	3–4	++++
Subpial glia	3–4	+/++	3–4	+++/++++
Capillaries	1–2	+/++	2–3	++/++++
Normal astrocytes	1–2	0/++	2–3	++/+++[b]
Hyperactive protoplasmic astrocytes	0	0	4	+/+++
Hyperactive fibrous astrocytes	0	0	4	+/+++[b]
Resting microglia	0	0	1–3	+/+++
Reactive microglia/macrophages	0	0	2–4	+/+++[c]

[a] Intensity is given by: 0, none; 1, faint; 2, weak; 3, moderate; 4, strong. Frequency is indicated by: 0, none; +, a few; ++, several; +++, some; ++++, many
[b] Frequency is greater in white matter than in gray
[c] Frequency is high (+++) in multiple sclerosis and amyotrophic lateral sclerosis tissue

Significant changes were observed in pathological tissue, with strongly hypertrophic glia appearing in affected areas. Changes in cytoskeletal organization accompaning pathological conditions are thought to reflect cellular adaptation to pathological stimuli. The "rules" governing changes in intermediate filament expression in such situations are only beginning to be understood (for reviews see [15, 22]). Vimentin is preferentially expressed in cells of mesenchymal origin, but it is known to be expressed in astrocytes along with GFAP. Embryologically, its expression in astrocytes precedes that of GFAP, but declines as the cells mature and GFAP expression increases [19, 20, 21, 23]. Following experimental stab wounds, vimentin immunoreactivity reappears in astrocytes at the margins of the wounds [20, 24]. Reactive astrocytes in these regions demonstrate [^{3}H]thymidine uptake [24]. Vimentin immunoreactivity also appears in astrocytes following permanent injury by experimentally induced ischemia [18]. These data, along with the known occurrence of vimentin in many neoplastic cells of mesenchymal origin as well as in gliomas, has led to the suggestion that vimentin expression is indicative of hyperplasia rather than hypertrophy [18, 20, 24]. Nevertheless, its increased expression in experimental induction of macrophage activity [3] and in reactive astrocytes would equally suggest a role related to stimulated activity.

In the pathological conditions described in this report, intense vimentin expression by astrocytes and microglia/macrophages was in hypertrophied, reactive structures. However, the techniques could not exclude the presence of mitotic profiles and, therefore, a relationship to hyperplasia. Vimentin up-regulation might be in response to unidentified cytokines or growth factors. Following experimental lesions in animals, vimentin mRNA and protein are up-regulated in injured areas, appearing in both reactive astrocytes and inflammatory cells [14]. However, even in such closely controlled conditions, distinctions have not yet been made between up-regulation due to hyperplasia and up-regulation due to hypertrophy. The clear association of vimentin-expressing reactive astrocytes and reactive microglia with AD senile plaques would suggest such structures as a possible source of a vimentin inducer.

Fig. 3A–L. Immunohistochemistry of vimentin in neurodegenerative diseased brains. **A** Activated protoplasmic astrocytes showing strong immunoreactivity for vimentin, and capillaries with moderate reactivity in mid-frontal cortex of a Pick's disease case. **B** Large, reactive astrocytes in the plaque of a multiple sclerosis case. **C** Double immunostaining for vimentin (dark profile, stained purple in the original slide) and β-amyloid protein (BAP; light profile, stained brown in the original). A vimentin-positive reactive astrocyte has processes surrounding and invading a senile plaque. **D** Vimentin-positive, fat-laden, reactive microglia (dark color) in the anterior column of ALS spinal cord counterstained with oil-red O (*light color*). Some of the microglia/macrophages are not stained by anti-vimentin antibody and are revealed only by Oil red O. **E** Double immunostaining with anti-vimentin (*purple, dark color*) and anti-BAP (*brown, light color*) antibodies in an Alzheimer's disease brain. A reactive microglia in a senile plaque can be seen to be vimentin positive. **F** Double immunostaining with anti-leukocyte common antigen (*purple, dark color*) and anti-BAP (*brown, light color*) in the same case of **E.** Compared with **E,** many resting microglia outside of the senile plaques and reactive microglia in the senile plaques can be seen. Electron micrographs showing the ultrastructure of an astrocyte (**G,** × 3348), a microglial cell (**I,** × 5740), and an endothelial cell (**K,** × 4836) from an Alzheimer's disease brain stained by anti-vimentin antibody. Magnification of the boxed areas are demonstrated in **H** (× 15066), **J** (× 25830) and **L** (× 14508). *N* = nucleus, *Cy* = vimentin-positive cytoskeleton in cell processes. *Bars* = 50 μm

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