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FIGURE LEGENDS

Fig. 1. AQP1 and AQP4 mRNA expression in human neural cells. The expression of (a) AQP1, (b) AQP4, and (c) G3PDH (an internal control) mRNA was studied in human neural cells by RT-PCR analysis. The lanes (1-11) represent (1) the frontal cerebral cortex (CBR), (2) cultured astrocytes (AS) without inclusion of the reverse transcription step (RT-), (3) cultured astrocytes (AS) with inclusion of the reverse transcription step (RT+), (4) cultured neuronal progenitor (NP) cells, (5) NTera2 teratocarcinoma-derived neurons (NTera2N), (6) Y79 retinoblastoma, (7) SK-N-SH neuroblastoma, (8) IMR-32 neuroblastoma, (9) U-373MG astrocytoma, (10) HeLa cervical carcinoma, and (11) HepG2 hepatocellular carcinoma. The DNA size marker (100 bp ladder) is shown on the left.

Fig. 2. AQP1 and AQP4 protein expression in HEK293 cells and human astrocytes in culture. (A) HEK293 cells. To verify the antibody specificity, ORF of the human AQP1 gene or the human AQP4 gene cloned in pcDNA4 vector was expressed in HEK293 cells, followed by processing for Western blot analysis using (a) anti-AQP1 antibody (H-55), (c) anti-AQP4 antibody (H-80), or (b, d) anti-HSP60 antibody. The lanes (1-6) represent (1,4) non-transfected HEK293 cells, (2,5) AQP1-expressing HEK293 cells, and (3,6) AQP4-expressing HEK293 cells. **(B) Astrocytes.** Human astrocytes were exposed for 48 hours to three distinct cytokines, followed by processing for Western blot analysis using (e) H-55, (f) H-80, (g) anti-GFAP antibody (GA5), (h) anti-IFI30 antibody, or (i) anti-HSP60 antibody. The lanes (7-10) represent (7) untreated cells, and the cells treated with (8) TNFα, (9) IFNγ and (10) IL-1β. Twenty microgram of protein was loaded on each lane. The identical blots were processed for relabeling several times with different antibodies. HSP60 indicates an internal control.

Fig. 3. AQP1 and AQP4 protein expression in MS and other neurological and psychiatric disease brain homogenates. The expression of (a) AQP1, (b) AQP4, (c) GFAP, and (d) HSP60 proteins was studied by Western blot analysis using H-55 and H-80 antibodies in brain homogenates prepared from either the frontal cerebral cortex (CBR) or the cerebellar cortex (CBL) of four MS and seven non-MS cases. The lanes (1-19) represent (1) MS#1 CBL, (2) MS#2 CBR, (3) MS#3 CBR, (4) MS#5 CBR, (5) a different specimen of MS#5 CBR, (6) MS#5 CBL, (7) a different specimen of MS#5 CBL, (8) PD#1 CBR, (9) PD#1 CBL, (10) ALS#1 CBR, (11) ALS#2 CBR, (12) ALS#2 CBL, (13) ALS#3 CBR, (14) ALS#3 CBL, (15) ALS#4 CBR, (16) ALS#4 CBL, (17) SCH#1 CBR, (18) DEP#1 CBR and (19) DEP#1 CBL. Abbreviations indicate PD, Parkinson disease; ALS, amyotrophic lateral sclerosis; SCH, schizophrenia; and DEP, depression. Sixty microgram of protein was loaded on each lane. The identical blots were processed for relabeling several times with different antibodies. HSP60 indicates an internal control.

Fig. 4. AQP1 and AQP4 immunoreactivities in human astrocytes in culture. Human astrocytes in culture were immunolabeled with (a) anti-AQP1 antibody (H-55) or (d) anti-AQP4 antibody (H-80), in combination with (b, e) anti-GFAP antibody (GA5). The panels (c, f) represent the merge of individual immunoreactions.

Fig. 5. AQP1 and AQP4 immunoreactivities in fibrillary astrocytes with highly-branched processes in MS and other neurological and psychiatric disease brains. The expression of AQP1 and AQP4 proteins was studied in MS and non-MS brains by immunohistochemistry. The panels (a-g) represent the following. (a) AQP1 (H-55), numerous astrocytes in chronic active demyelinating lesion in the frontal cerebral cortex of MS#1. (b) AQP1 (H-55), a fibrillary astrocyte in the unaffected cortex adjacent to old infarct lesion in the frontal cerebral cortex. (c) Double labeling of AQP1 (H-55, red) and GFAP (GA5, brown), a fibrillary astrocyte in chronic active

demyelinating lesion in the occipital cerebral cortex of MS#1. (d) AQP4 (H-80), astrogliosis in the rim of necrotic core of old infarct in the temporal cerebral cortex. (e) AQP4 (H-80), two fibrillary astrocytes in the unaffected cortex adjacent to old infarct lesion in the temporal cerebral cortex. (f) Double labeling of AQP4 (H-80, red) and GFAP (GA5, brown), several fibrillary astrocytes in chronic active demyelinating lesion in the occipital cerebral cortex of MS#1. (g) AQP1 (H-55), no obvious immnoreactivity in the frontal cerebral cortex of a neurologically normal subject. (h) AQP4 (H-80), the pia and subpial astrocytes, the same region as **g**. (i) Double labeling of AQP1 (1/22, red) and AQP4 (H-80, brown), several fibrillary astrocytes in the frontal cerebral cortex of a patient with schizophrenia.

Fig. 6. AQP1 and AQP4 immunoreactivities in gliotic tissues in MS brains. The expression of AQP1 and AQP4 proteins was studied in the optic nerve and the spinal cord by immunohistochemistry. The panels (a-f) represent the following. (a) GFAP (rabbit polyclonal antibody), gliotic tissues in chronic active demyelinating lesion in the optic nerve of MS#2. (b) AQP1 (H-55), the same region as **a**. (c) AQP4 (H-80), the same region as **a**. (d) GFAP (rabbit polyclonal antibody), gliotic tissues in the posterior funiculus of the spinal cord of MS#3. (e) AQP1 (H-55), the same region as **d**. (f) AQP4 (H-80), the same region as **d**.

Fig. 7. Absence of AQP1 and AQP4 immunoreactivities in macrophages, neurons, and oligodendrocyte cell bodies in MS and other neurological and psychiatric disease brains. The expression of AQP1 and AQP4 proteins was studied in MS and non-MS brains by immunohistochemistry. The panels (a-f) represent the following. (a) AQP1 (H-55), numerous perivascular macrophages in chronic active demyelinating lesion in the frontal cerebral cortex of MS#3. (b) AQP1 (H-55), surviving oligodendrocytes in chronic active demyelinating lesion in the frontal cerebral cortex of MS#2. (c) AQP1 (H-55), motor neurons in the spinal cord of MS#2. (d) AQP4 (H-80),

foamy macrophages in acute necrotic lesion of cerebral infarction in the parietal cerebral cortex. (e) AQP4 (H-80), surviving oligodendrocytes in chronic active demyelinating lesion in the parietal cerebral cortex of MS#4. (f) AQP4 (H-80), pontine neurons in the brain stem of MS#2.

Table 1 Primary antibodies utilized for immunohistochemistry and Western blot analysis

Antibodies (ID)	Suppliers	Code	Origin	Antigen utilized for raising antibodies	Concentration used for immunohistochemistry	Concentration used for Western blotting
AQP1 (H-55)	Santa Cruz Biotechnology	sc-20810	rabbit	a peptide covering amino acid residues 215-269 of human AQP1	0.4 μg/ml	0.2 μg/ml
AQP1 (1/22)	Santa Cruz Biotechnology	sc-32737	mouse	a peptide covering amino acid residues 249-269 of rat AQP1	8 μg/ml	NA
AQP4 (H-80)	Santa Cruz Biotechnology	sc-20812	rabbit	a peptide covering amino acid residues 244-323 of human AQP4	0.4 μg/ml	0.2 μg/ml
GFAP	Dako	N1506	rabbit	GFAP purified from bovine spinal cord	prediluted	NA
GFAP (GA5)	Nichirei	422261	mouse	GFAP purified from swine spinal cord	prediluted	further diluted at 1: 1000
MBP	Dako	N1564	rabbit	MBP purified from human brain	prediluted	NA
NF (2F11)	Nichirei	412551	mouse	NF purified from human brain	prediluted	NA
CD68 (KP1)	Dako	N1577	mouse	lysosomal granules of human lung macrophages	prediluted	NA
IFI30 (T-18)	Santa Cruz Biotechnology	sc-21827	goat	a peptide mapping within an internal region of human IFI30 (GILT)	NA	0.2 μg/ml
HSP60 (N-20)	Santa Cruz Biotechnology	sc-1052	goat	a peptide mapping at the N-terminus of human HSP60	NA	0.1 μg/ml

Abbreviations: AQP1, aquaporin-1; AQP4, aquaporin-4; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; NF, neurofilament; IFI30, interferon gamma-inducible protein 30; GILT, gamma interferon-inducible thiol reductase; HSP60, 60-kDa heat shock protein; and NA, not applied.

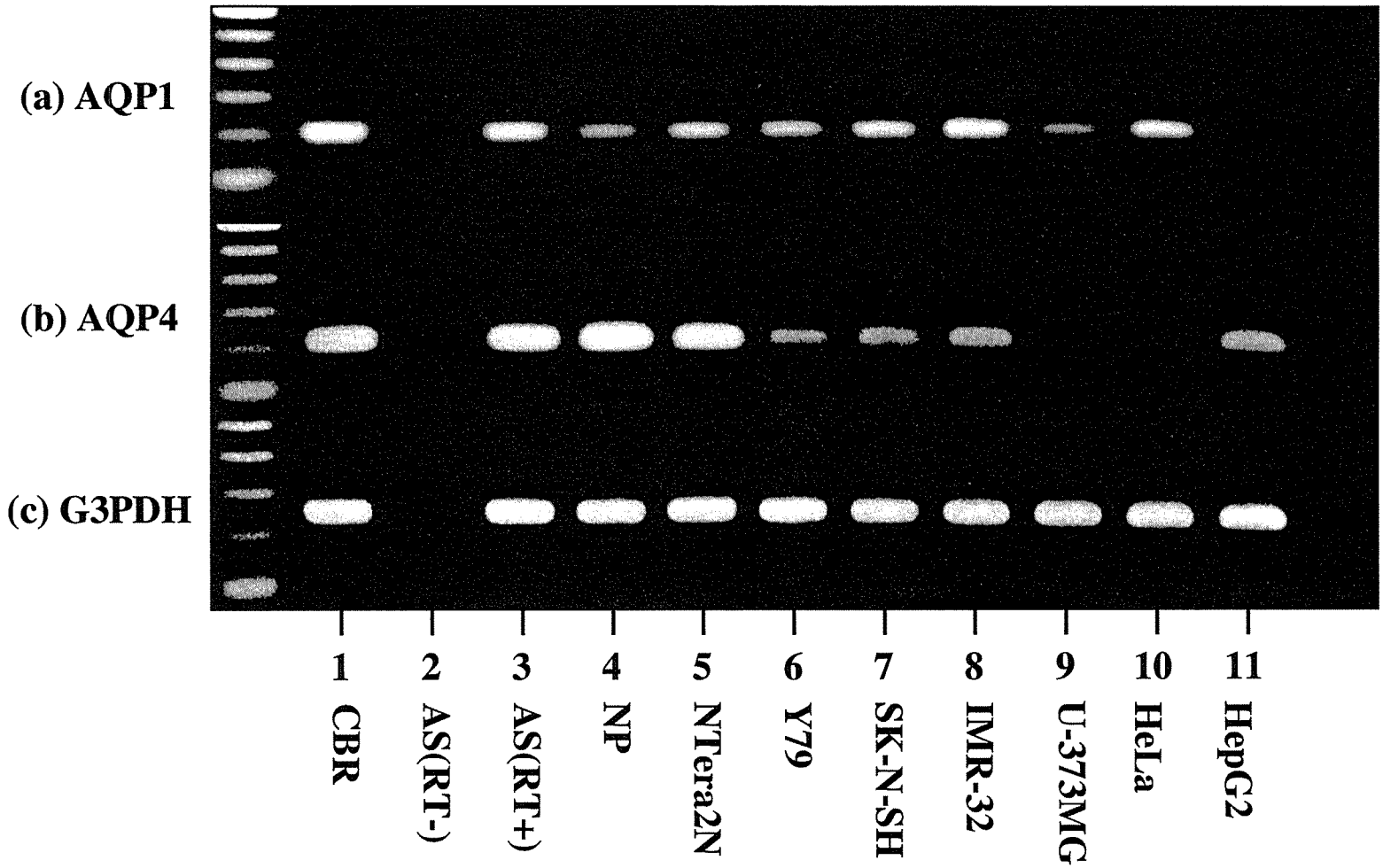
(a) AQP1
(b) AQP4
(c) G3PDH
1 CBR
2 AS(RT-)
3 AS(RT+)
4 NP
5 NTera2N
6 Y79
7 SK-N-SH
8 IMR-32
9 U-373MG
10 HeLa
11 HepG2

Fig. 1

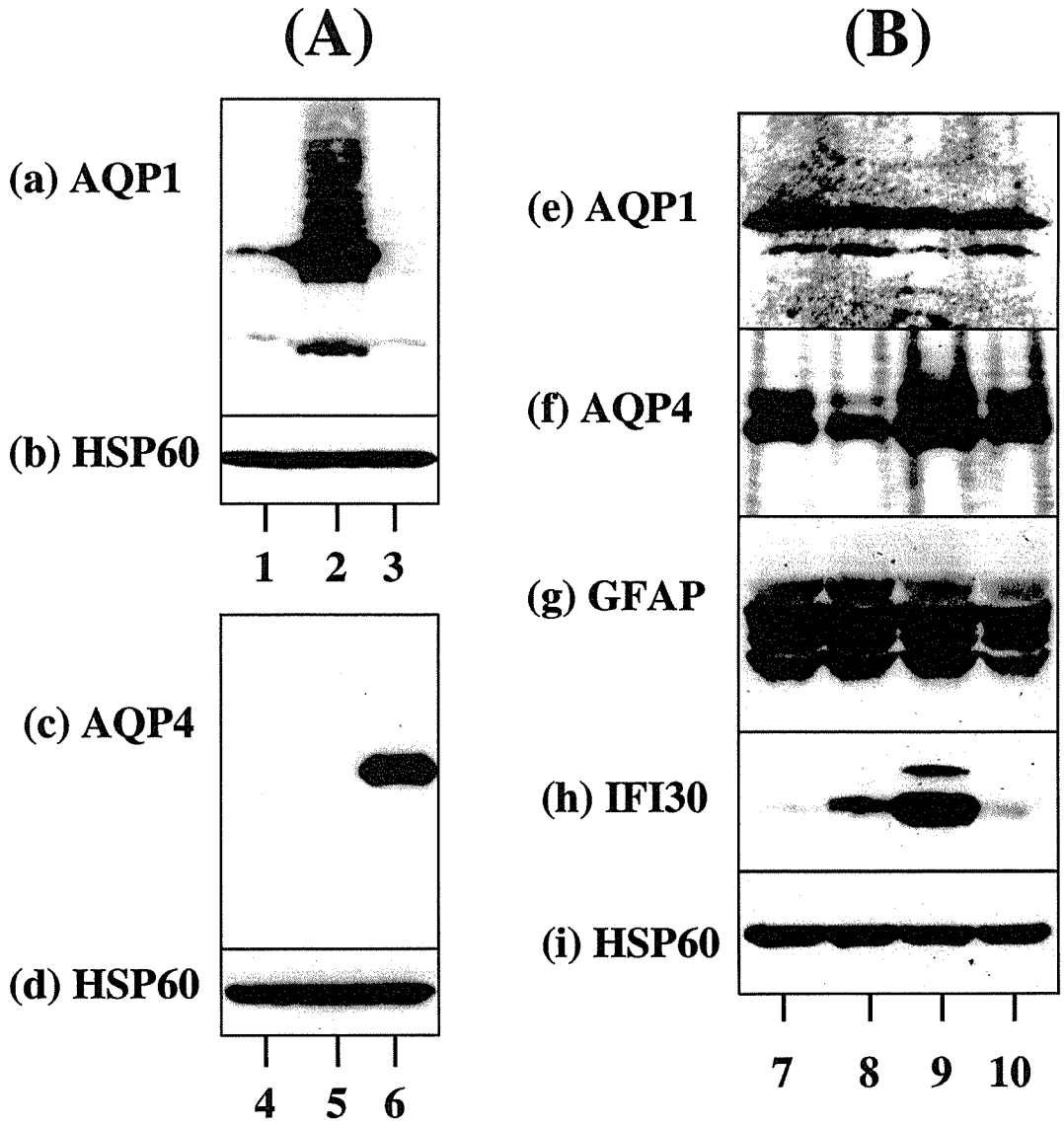
(A)
(B)
(a) AQP1
(b) HSP60
1 2 3
(c) AQP4
(d) HSP60
4 5 6
(e) AQP1
(f) AQP4
(g) GFAP
(h) IFI30
(i) HSP60
7 8 9 10

Fig. 2

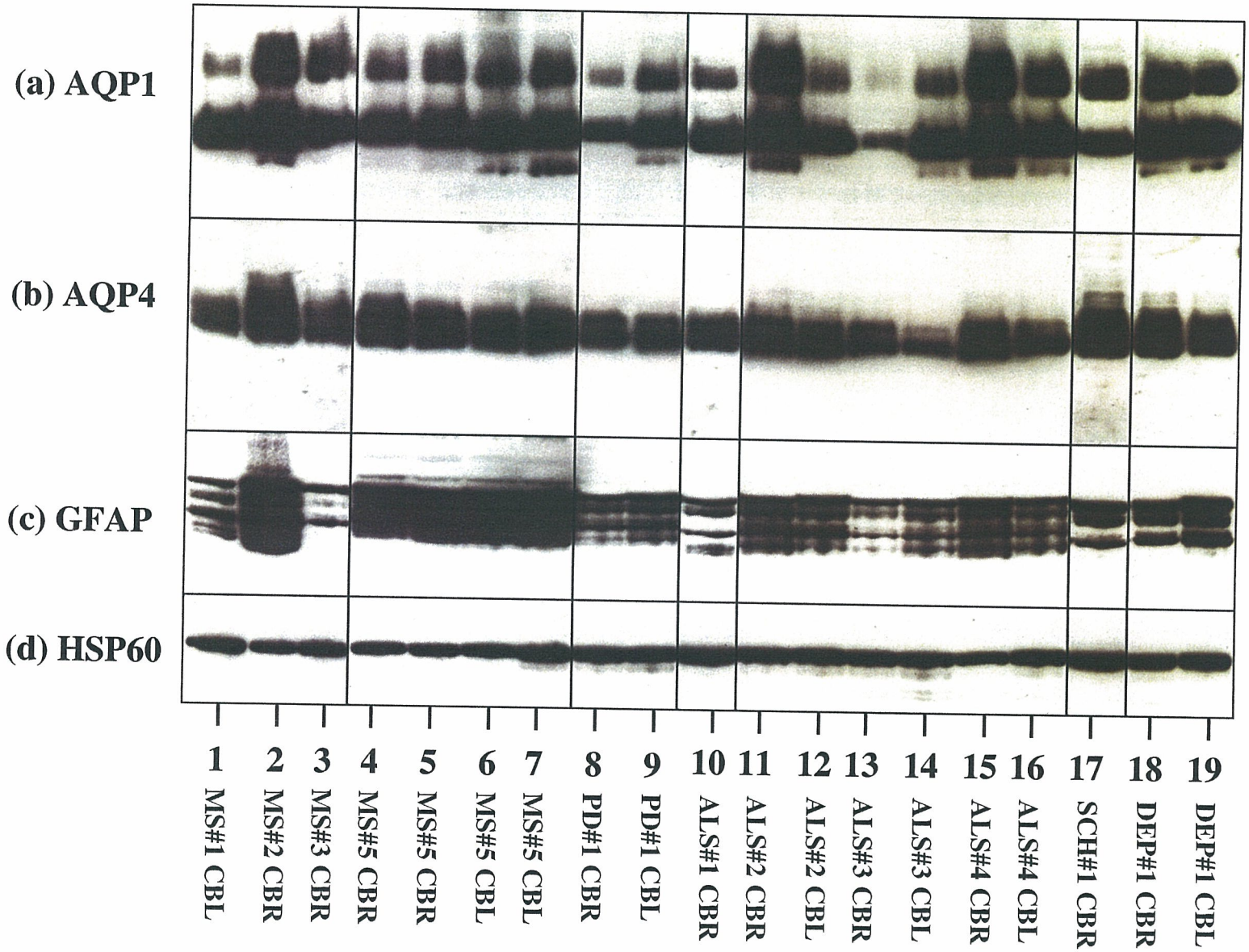
(a) AQP1
(b) AQP4
(c) GFAP
(d) HSP60
1 MS#1 CBL
2 MS#2 CBR
3 MS#3 CBR
4 MS#5 CBR
5 MS#5 CBR
6 MS#5 CBL
7 MS#5 CBL
8 PD#1 CBR
9 PD#1 CBL
10 ALS#1 CBR
11 ALS#2 CBR
12 ALS#2 CBL
13 ALS#3 CBR
14 ALS#3 CBL
15 ALS#4 CBR
16 ALS#4 CBL
17 SCH#1 CBR
18 DEP#1 CBR
19 DEP#1 CBL

Fig. 3

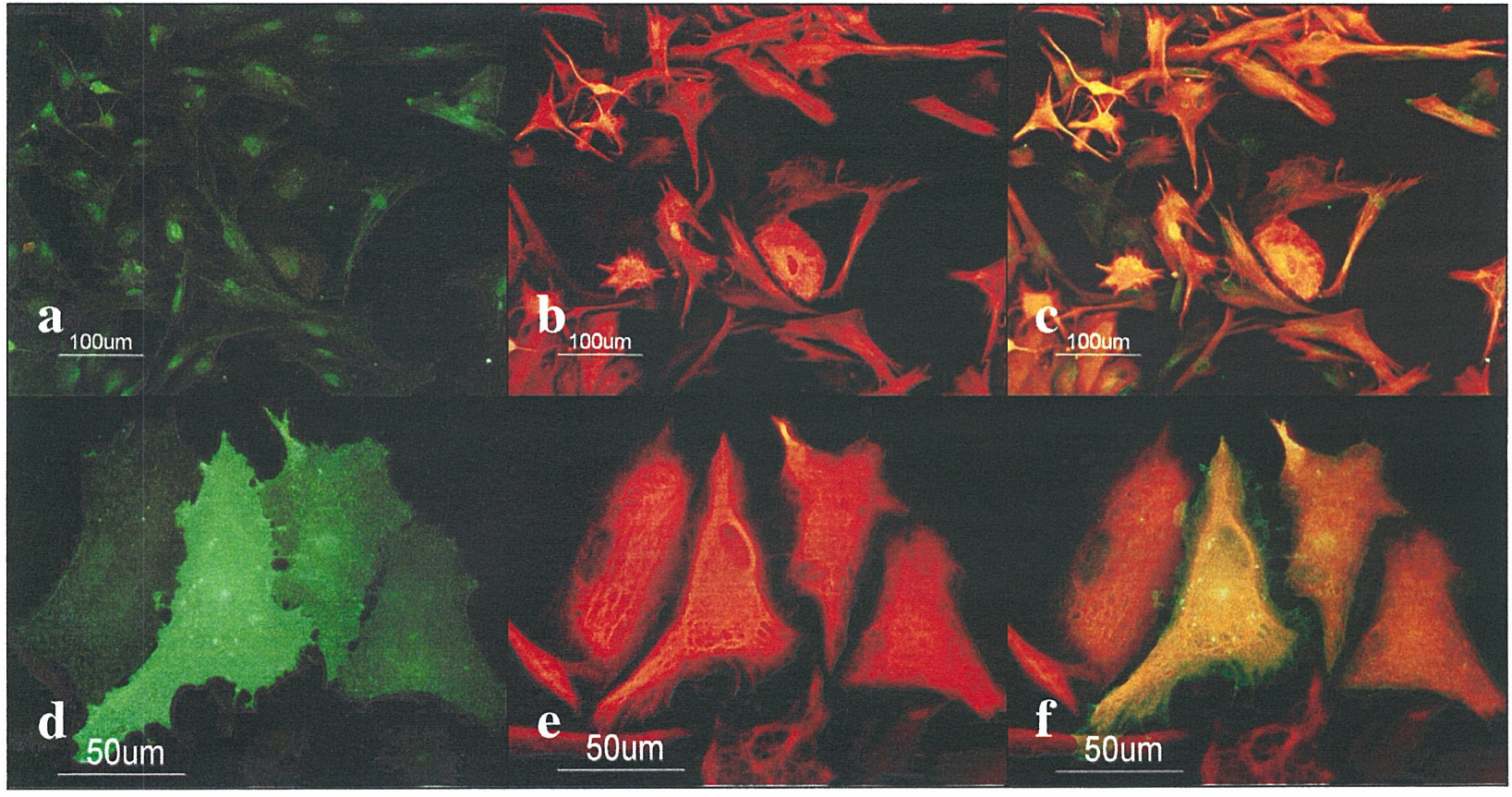

Fig. 4

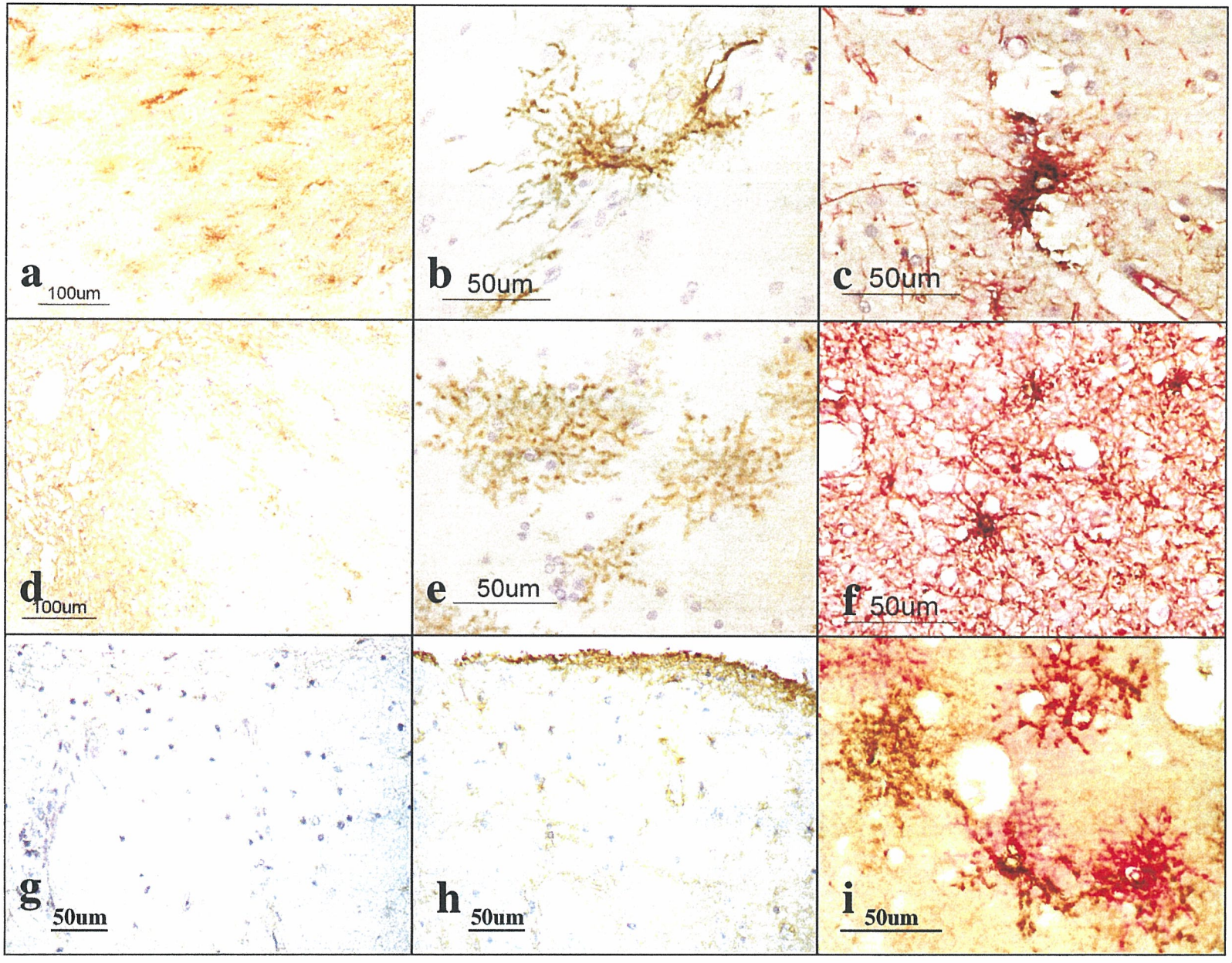

Fig. 5

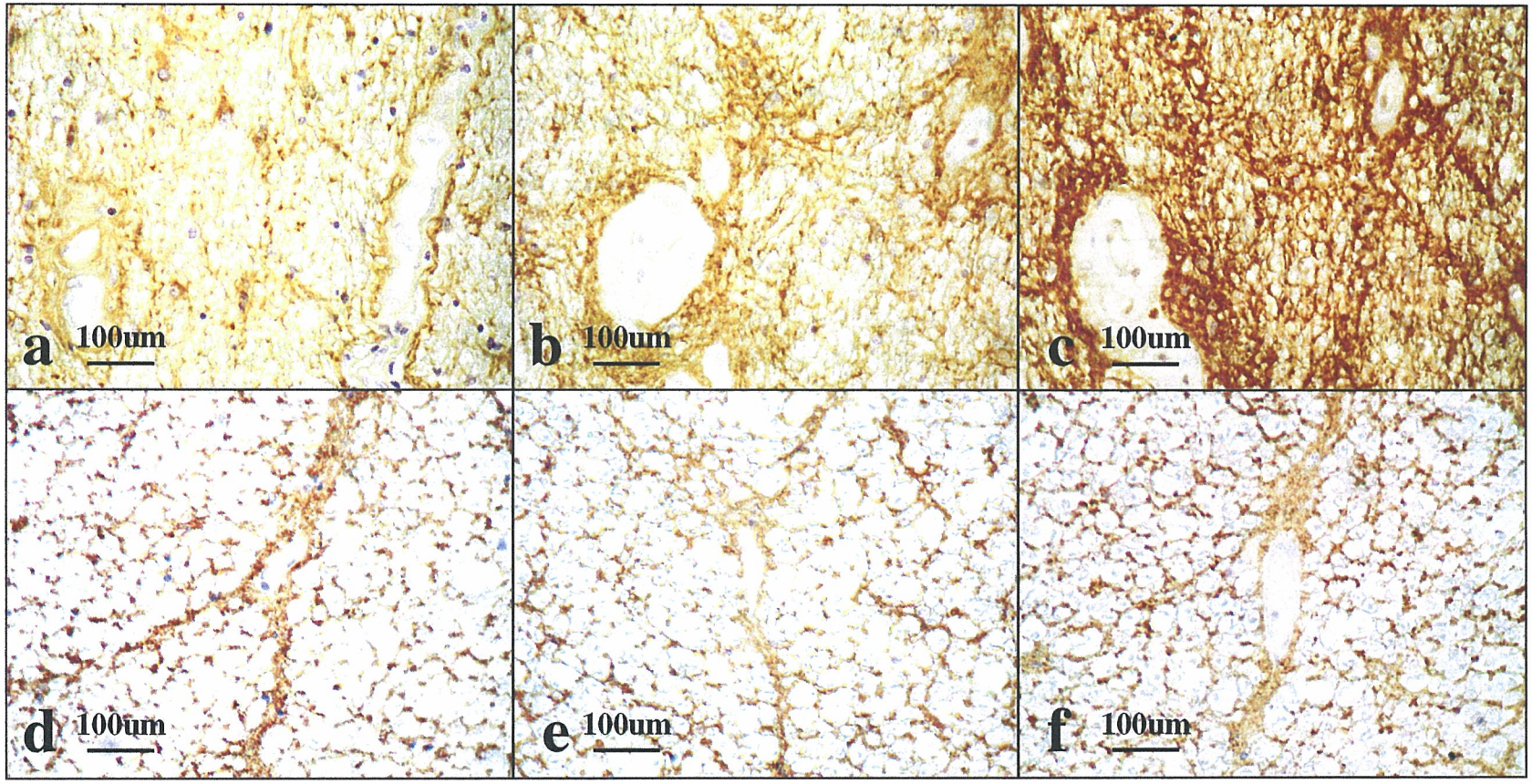

Fig. 6

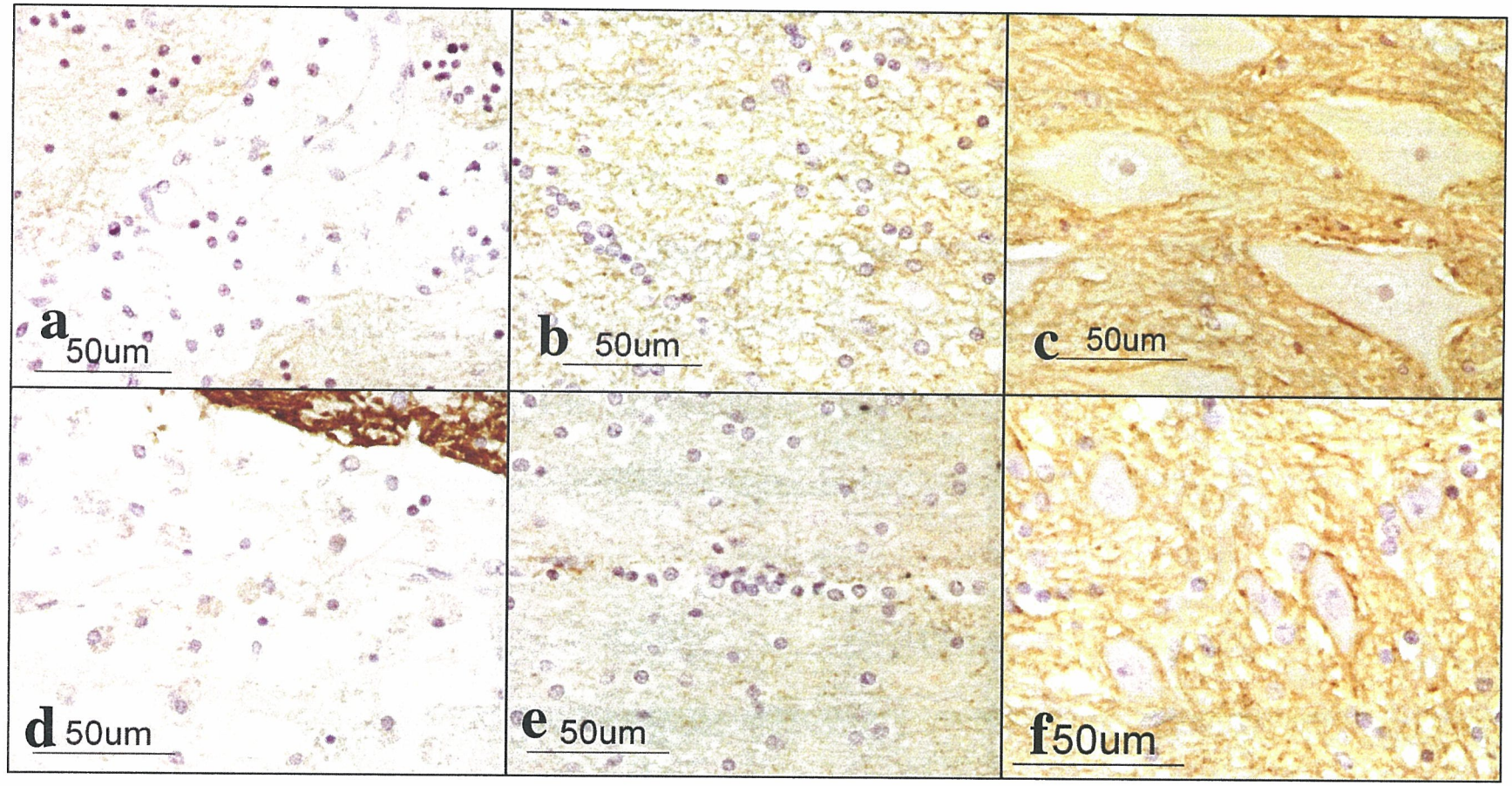

Fig. 7