

Review

Angiogenesis in the central nervous system: a role for vascular endothelial growth factor/vascular permeability factor and tenascin-C. Common molecular effectors in cerebral neoplastic and non-neoplastic “angiogenic diseases”

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Summary. Human pathological conditions of the central nervous system (CNS) associated with angiogenesis (i.e. neovascularization) include neoplastic, as well as infectious, ischemic, and traumatic processes. Upregulation of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and tenascin-C (TN-C) is spatially and temporally related to neovascularization. Spatially, VEGF/VPF and TN-C are both found at the site of neovascularization, but they are not detected in areas of normal brain or in areas without neovascularization. Temporally, VEGF/VPF and TN-C are found at the peak of angiogenesis and are not detected when angiogenesis had ceased.

Key words: Angiogenesis, Central nervous system, Tenascin, Vascular endothelial growth factor/vascular permeability factor

Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF)

Angiogenesis, i.e. neovascularization, is a complex biological process whose regulatory mechanisms are not completely understood. Formation of new vessels occurs not only during embryogenesis, wound healing and regeneration, but also in pathological processes, e.g. neoplasia, diabetic retinopathy and arthritis (Folkman, 1995; Yancopoulos et al., 1998). VEGF/VPF (Senger et al., 1983; Nicosia, 1998) is a hypoxia-inducible,

(Shweiki et al., 1992) secreted endothelial cell mitogen, (Nicosia, 1998) which has been shown to increase microvascular permeability and endothelial fenestration (Senger et al., 1983; Roberts and Palade, 1995). This ~45 kDa heparin-binding glycoprotein dimer contains two subunits of equivalent mass and is structurally homologous to platelet-derived growth factor (Nicosia, 1998). Of the four different isoforms arising from alternative mRNA splicing (VEGF/VPF₁₂₁, 165, 189, 206), VEGF/VPF₁₆₅ is predominantly expressed (Ferrara et al., 1991). The shorter forms are diffusible whereas the longer ones are bound to the extracellular matrix (ECM) (Ferrara et al., 1991). VEGF/VPF is secreted by a variety of cell types and is angiogenic *in vivo* (Ferrara et al., 1991; Claffey and Robinson, 1996; Nicosia, 1998). Although VEGF/VPF is thought to be a specific endothelial cell mitogen, receptors for VEGF/VPF have been demonstrated on smooth muscle cells (Brown et al., 1997). The two human VEGF/VPF receptors: flt-1/VEGFR-1 (De Vries et al., 1992) and KDR/VEGFR-2 (Terman et al., 1992) are widely distributed on endothelial cells (Millauer et al., 1993) while VEGFR-3 is specifically distributed on lymphatic endothelial cells (Jeltsch et al., 1997). Moreover, a novel receptor that binds VEGF/VPF₁₆₅ but not VEGF/VPF₁₂₁ was described and found to be identical to human neuropilin-1, a receptor for the collapsin/semaphorin family that mediates neuronal cell guidance (Soker et al., 1998). VEGF/VPF currently appears to be the principal mediator and a potent inducer of angiogenesis during normal physiological processes such as vascular development (Nicosia, 1988; Ferrara et al., 1991; Plate et al., 1992; Jakeman et al., 1993; Millauer et al., 1993; Breier et al., 1995; Zagzag, 1995; Claffey and Robinson, 1996; Brown et al., 1997; Nicosia, 1998) and in

inflammatory and neoplastic pathologies outside the CNS (Jakeman et al., 1993; Millauer et al., 1993; Breier et al., 1995; Dvorak et al., 1995). Hypoxia-inducible factor-1 (HIF-1), a heterodimeric basic-helix-loop-helix-PAS (bHLH-PAS) transcription factor composed of HIF-1 α and HIF-1 β subunits, plays an essential role in oxygen homeostasis (Wang and Semenza, 1995; Wang et al., 1995; Iyer et al., 1998). HIF-1 β , which is also known as the aryl hydrocarbon receptor nuclear translocator (Hoffman et al., 1991), can dimerize with several different bHLH-PAS transcription factors. In contrast the HIF-1 α subunit is unique to HIF-1. Its expression increases as cellular O₂ concentration decreases, and determines the level of HIF-1 activity (Wang et al., 1995; Jiang et al., 1996; Semenza et al., 1996). HIF-1 activates a large battery of genes whose protein products function either to increase O₂ availability or to allow metabolic adaptation to O₂ deprivation (Semenza, 1998). Included among these are genes encoding VEGF/VPF erythropoietin, glucose transporters, glycolytic enzymes, insulin-like growth factor 2 (IGF2), and IGF binding proteins-1, -2, and -3 (Semenza et al., 1996; Iyer et al., 1998; Semenza, 1998; Tazuke et al., 1998; Feldser et al.,

1999). Such genes share the presence of hypoxia-response elements, which contain binding sites for HIF-1. Upon reoxygenation, HIF-1 is rapidly degraded, both in cultured cells and *in vivo* (Wang et al., 1995; Huang et al., 1996). Hypoxia or iron chelation prevents ubiquitination of HIF-1. The interaction between pVHL and HIF-1 is regulated the binding of pVHL to a hydroxylated proline residue of HIF-1 (Ivan et al., 2001; Jaakkola et al., 2001). In addition to hypoxia, the regulation of VEGF/VPF expression may involve diverse mechanisms including activated oncogenes, mutant or deleted tumor suppressor genes, and cytokine activation (Claffey and Robinson, 1996).

Tenascin-C

TN-C is a large complex secreted protein of the ECM which is expressed in developing brain, cartilage and mesenchyme and is re-expressed in tumors, wound healing and inflammation (Erickson, 1993; Redick and Schwarzbauer, 1995) where there is remodeling of the ECM (Erickson, 1993). It has a characteristic six-armed quaternary structure (hexabrachion) linked to a central

Table 1. VEGF/VPF and TN-C expression in neoplastic angiogenesis.

| AGE (years) | SEX | SITE | VEGF/VPF | | TENASCIN-C | | | | |
|----------------|-----|-------------------|------------|-------------|------------|-------------|---------|--------|--|
| | | | ISH | | ISH | | IHC | | |
| | | | Vasc Cells | Tumor Cells | Vasc Cells | Tumor Cells | PV | IC | |
| GBM | | | | | | | | | |
| 2 | F | L. Frontal Lobe | -/- | + /+++ | + /+++ | + /+++ | + /+++ | + /+++ | |
| 39 | F | L. Parietal Lobe | -/- | ++ /+++ | + /+++ | - /+ | + /+ | - /+ | |
| 47 | M | L. Parietal Lobe | -/- | + /+++ | + /+++ | + /+ | + /+++ | + /+++ | |
| 48 | F | R. Occipital Lobe | -/- | + /+++ | + /+++ | - /+++ | + /+++ | - /+++ | |
| 55 | M | R. Frontal Lobe | -/- | ++ /+++ | + /+ | + /+++ | + /+++ | + /+++ | |
| JPA | | | | | | | | | |
| 4 | M | Cerebellum | -/- | + /++ | + /+++ | - /+ | + /+++ | - /+ | |
| 5 | F | Cerebellum | -/- | - /++ | - /+ | - | - /+ | - /- | |
| 8 | M | Cerebellum | -/- | - /- | - /+++ | - /+ | - /+++ | - /+ | |
| 8 | F | Cerebellum | -/- | - /- | - /+ | - | - /+++ | + /+ | |
| 12 | F | Cerebellum | -/- | + /+++ | - /+ | - | - /+ | - /+ | |
| 13 | F | Lateral Ventricle | -/- | + /+ | - | - | - /+ | - /- | |
| HB | | | | | | | | | |
| 15^ | M | Cerebellum | -/- | + /+++ | + /+++ | - /+ | + /+++ | - /+ | |
| 17^ | M | Spinal Cord | -/- | ++ /+++ | + /+++ | - /+ | ++ /+++ | - /+ | |
| 18~ | F | Cerebellum | -/- | ++ /+++ | + /+++ | - /+ | + /+++ | - /+ | |
| 28 | F | Cerebellum | -/- | + /+++ | - /+++ | - | - /++ | + /+++ | |
| 38 | F | Spinal Cord | -/- | ++ /+++ | + /+++ | - /+ | ++ /+++ | - /++ | |
| 39 | M | Cerebellum | -/- | ++ /+++ | + /+++ | - /+ | + /+++ | - /+ | |
| 45 | M | Cerebellum | -/- | ++ /+++ | + /+++ | - /+ | + /++ | + /+++ | |

ISH: in situ hybridization; IHC: immunohistochemistry; vasc: vascular; PV: perivascular; IC: intercellular; GBM: glioblastoma multiforme; JPA: juvenile pilocytic astrocytoma; HB: hemangioblastoma; \wedge : same patient; \sim : patient with Von Hippel-Lindau; -: not detected; +: weak; ++: moderate; +++: strong. The tumors included 5 GBMs (astrocytoma, WHO Grade IV/IV), 6 JPAs (astrocytoma WHO Grade I/IV (Kleihues et al., 1993)), and 7 hemangioblastomas. We assessed the presence of vascular hyperplasia in each case, taking into account the following three histological criteria: 1) increased vascular density, 2) increased number of vascular cell layers, and 3) plump endothelial cells (Brem et al., 1972). Three out of 6 JPAs and 4 out of 5 GBMs showed glomeruloid vascular complexes. One GBM without vascular hyperplasia was classified as such because of the presence of necrosis. Two JPAs cases showed tumor infarction (Giannini and Scheithauer, 1997). The 7 hemangioblastomas were highly vascular but had variable cell density with highly cellular and paucicellular regions. Four samples of histologically normal brain removed in the course of surgical exposure were used as controls. When present, normal tissue adjacent to the lesions was used as internal controls. There was no VEGF/VPF mRNA in normal brain vasculature and scant signal was detected in normal cerebral cortex but not in white matter in the 4 normal controls.

knob formed by disulfide links of cysteines in the N-terminal ends of the six polypeptide arms (Erickson, 1993). In addition, TN-C consists of epidermal growth factor-like and fibronectin-type III repeats, and a fibrinogen-like region at the carboxyl terminus. At least

2 structurally and functionally different human TN isoforms (~200 and 300kDa) are generated by alternative splicing, with seven type III repeats being included or omitted in the mRNA (Erickson, 1993). Knockout of TN-C expression in mice had no major phenotypic

Table 2. VEGF/VPF and TN-C expression in non-neoplastic angiogenesis.

| | AGE (years) | SEX | SITE (LOBE) | INTERVAL^ | VEGF/VPF | | Tenascin-C | | | |
|-----------------------|----------------|-----|----------------------|-----------|-------------------|----------------------|-------------------|----------------------|--------|-------|
| | | | | | ISH | | ISH | | IHC | |
| | | | | | Vascular Cells | Nonvascular Cells | Vascular Cells | Nonvascular Cells | PV | IC |
| Abscess ¹ | | | | | | | | | | |
| | 69 | F | R. temoro-parietal | 1day | -/+++ | -/+++ | + /++ | + /+ | + /+++ | + /++ |
| | 9 | F | L. temporo-parietal | 7 days | + /++ | + /+++ | + /+++ | - /+ | - /++ | - /+ |
| | 45 | M | R. occipital | 14 days | - /+ | + /+++ | + /+++ | - /++ | - /+++ | - /++ |
| | 56 | M | L. lateral ventricle | 21 days | - /+ | + /+++ | + /++ | - /+ | + /++ | - /+ |
| | 12 | M | L. frontal | 30 days | - /++ | - /++ | + /+++ | - /++ | + /+++ | + /++ |
| | 32 | M | L. frontal | 34 days | - /- | + /+ | - /++ | - /+ | - /+++ | - /++ |
| Infarcts ² | | | | | | | | | | |
| | 33 | M | R. temporal | 1 day | - /+ | - /+ | + /+ | - /+ | + /++ | - /+ |
| | 23 | M | R. temporal | 3 days | + /++ | - /+ | + /+ | + /+++ | + /+ | - /+ |
| | 47 | F | L. frontal | 5 days | + /+++ | + /+++ | + /++ | + /+++ | - /++ | - /++ |
| | 61 | M | L. occipital | 7 days | + /++ | - /+++ | + /+++ | + /+++ | - /+++ | - /++ |
| Trauma ³ | | | | | | | | | | |
| | 35 | M | L. temporo-parietal | 2 days | - /+ | - /+ | + /+ | + /+ | - /+ | - /- |
| | 40 | M | R. frontal | 7 days | - /++ | + /+++ | + /+++ | + /++ | + /++ | + /+ |
| | 53 | M | R. frontal | 9 days | + /++ | + /+++ | + /+++ | + /++ | + /++ | + /++ |
| | 57 | M | L. frontal | 12 days | + /+++ | + /+++ | + /++ | + /++ | + /+++ | + /++ |
| | 25 | M | L. frontal | 14 days | + /++ | - /+ | - /+ | + /++ | + /+++ | + /++ |
| | 6 | F | R. frontal | 1825 days | - /- | - /- | - /- | - /- | - /+ | - /+ |
| SDH ⁴ | | | | | | | | | | |
| | 73 | M | Bilateral convexity | 1 day | - /+ | - | + /+++ | + /+ | - /++ | - /+ |
| | 70 | M | R.frontal-parietal | 14 days | - /+ | + /+++ | + /++ | - /+ | - /++ | - /++ |
| | 92 | F | L. frontal | 42 days | - /++ | - /+ | + /+ | - /+ | + /+++ | + /++ |
| | 79 | M | Bilateral convexity | 84 days | + /+ | - /+ | + /+ | - /+ | + /++ | - /+ |
| | 50 | M | L. parietal | 120 days | - /- | - /- | - / | - /- | - /- | - /- |

¹: for abscesses and subdural hematomas (SDH) the interval is the time between the onset of symptoms and the surgical procedure. By contrast, for infarcts and traumas, it indicates the time between the onset of the clinical symptoms or head injury and the surgical procedure; ISH: in situ hybridization; IHC: immunohistochemistry; PV: perivascular; IC: intercellular; -: not detected; +: weak; ++: moderate; +++: strong pathological event and the surgical procedure; ie vascular occlusion or head injury; ISH: in situ hybridization; IHC: immunohistochemistry; PV: perivascular; IC: intercellular; -: not detected; +: weak; ++: moderate; +++: strong

¹: In each case, the wall of an organizing cerebral abscess i.e. inflamed "granulation tissue" with variable matrix deposition around a necrotic center with marked neovascularization was seen (Hardman, 1979). Organisms identified by gram stain and culture were *Nocardia* spp (two cases), *Streptococcus intermedius* and *Acinetobacter* in one. In 2 cases no organisms were found.

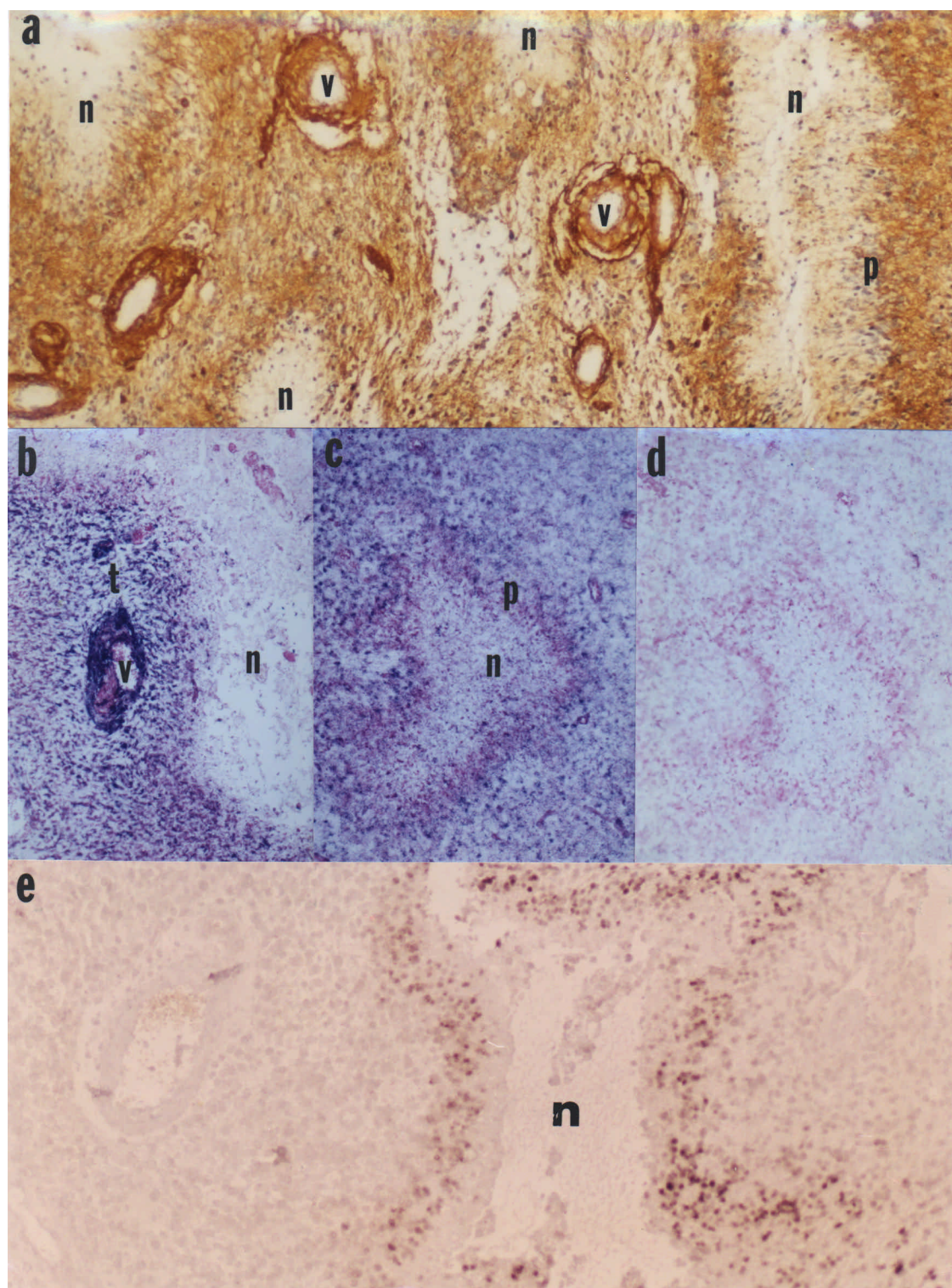
²Pathological examination (Garcia, 1992) of the four cerebral infarcts reveals hemorrhagic (3 cases) and non hemorrhagic "bland" (1 case) infarcts. Cases included a 24 hour old hemorrhagic infarct (due to cocaine abuse), a 3 day old arteriovenous malformation associated-hemorrhagic infarct, a 5 day old bland infarct and a 7 day old hemorrhagic infarct thought to be an intratumoral hemorrhage. This case showed luxurious vascular proliferation admixed with histiocytic cells. VEGF/VPF signal was strong in 5 and 7 day old infarcts. TN-C immunostaining was scant 1 and 3 days after the hypoxic injury, was more evident after 5 days and marked 7 days after onset of hypoxia/ischemia.

³: Surgical specimens were obtained 2, 7, 9, 12, and 14 days after blunt trauma to the head (Cancilla et al., 1979). Vascular proliferation was not detected in the lesion operated on 2 days after trauma, but was seen with increasing intensity on days 7 and 9 and was marked between days 12 and 14. The sixth patient was a 6 year old girl who suffered from medically refractory seizures 5 years after a car accident. The specimen obtained from this patient showed features consistent with old remote contusion with cavitation and gliosis. VEGF/VPF progressively increased in 7, 9, and 12 day old contusions but was less pronounced by day 14 and was not detected in the 5 year old injury. TN-C expression was stronger in the recent infarct as compared to TN-C expression accompanying the more chronic changes. Perivascular immunostaining was more pronounced in the areas of prominent neovascularization while the intervascular staining was variable. TN-C immunostaining increased from days 2 to 7, was maximal on day 9, was similar on days 12 and 14, and was not detected in the case obtained 5 years after injury. Thus, in cerebral contusions perivascular staining correlated with the extent of neovascularization and was not detected in the remote contusion.

⁴: Five patients, were operated upon to remove subdural hematomas from 1 to 120 days after onset of clinical symptoms. Pathological examination (Hardman, 1979) obtained 1 and 14 days after onset of clinical symptomatology showed well developed sinusoids within the clot. The specimens obtained 42 and 84 days after clinical onset showed recent hemorrhage within a more chronic membrane i.e. less abundant vascularization and more prominent matrix deposition. The case obtained over 4 months after beginning of symptoms revealed a hyalinized membrane with hemosiderin laden macrophages with no discernable vascular channels. In the case where tissue was obtained 5 years after injury no signal was found (data not shown).

abnormality (Saga et al., 1992; Forsberg et al., 1996) e.g. nerve regeneration and healing of cutaneous wounds is the same as in controls (Forsberg et al., 1996). Nevertheless, TN-C is believed to be important for

several cellular processes including adhesion, migration, and proliferation of cells (Erickson, 1993). A variety of cell types including astrocytes (Bourdon et al., 1983; Grumet et al., 1985; Dorries et al., 1993; Brodkey et al.,



1995; Zagzag et al., 1995, 1996) and vascular cells (Schor et al., 1991; Mackie et al., 1992; Webersinke et al., 1992; Canfield and Schor, 1995; Hahn et al., 1995; Zagzag et al., 1996) express TN-C *in vitro* and *in vivo*.

Angiogenesis in the central nervous system

Angiogenesis plays a critical pathogenetic role in many pathological processes of the CNS. It is crucial for brain tumor growth (Zagzag et al., 1988; Cheng et al., 1996). The vascular proliferation associated with gliomas is well recognized (Burger et al., 1985) and is one of the criteria used for their grading (Damas-Duport et al., 1988). Neovascularization often correlates with biological aggressiveness and degree of malignancy of brain tumors as well as clinical recurrence, and inversely with post-operative survival of patients with anaplastic astrocytomas (Burger et al., 1985; Damas-Duport et al., 1988). Infiltration of malignant tumors in the brain can follow vascular channels (Scherer, 1940; Zagzag et al., 1988, 2000). Newly formed brain tumor blood vessels with defective blood-brain barrier (Zagzag et al., 1988, 1989; Del Maestro et al., 1990) are responsible for the contrast enhancement of brain tumors (Zagzag et al., 1989). They are associated with an increased risk of intratumoral hemorrhage (Liwnicz et al., 1987) and contribute to the pathogenesis of tumor-associated edema (Zagzag et al., 1998, 1989; Del Maestro et al., 1990). Like high grade gliomas, hemangioblastomas are highly vascular neoplasms. They are formed by two cellular components i.e. vascular cells and "stromal" cells. It has been suggested by several investigators that the stromal cells are the "main tumor cells" (Castaigne et al., 1968) and the vascular component is the result of an exuberant "reactive" vascular proliferation.

In cerebral abscesses, (Britt and Enzmann, 1983)

two main stages exist. These are cerebritis and encapsulation. Each of these two stages can be subdivided in two, i.e. early and late substages. Early cerebritis (days 1-3) is associated with the spread of organisms across the injured vascular wall and with early necrosis, vascular congestion, petechial hemorrhages, microthromboses, perivascular fibrinous exudates and acute inflammation. Even at this early stage the endothelial cells swell. However, definite neovascularization is usually detected in the late cerebritis stage (days 4-9) when the necrotic purulent center is surrounded by a narrow irregular layer of granulation tissue infiltrated by neutrophils, lymphocytes and some macrophages often cuffing the perivascular spaces. At this stage, endothelial cells show marked hypertrophy and hyperplasia including mitoses and there is increased capillary density. Subsequently (days 10-13), matrix deposition around numerous newly formed blood vessels results in an early poorly defined developing abscess wall. As time passes (day 14 and later), the wall becomes firmer and is well demarcated from the surrounding edematous brain. Thus, neovascularization plays a major role in the organization of the wall of the abscess from matrix deposition to encapsulation.

Cerebral infarcts (Liu, 1988; Garcia, 1992) and traumas (Mitchell et al., 1978; Cancilla et al., 1979; Hardman, 1979) are histologically similar. However, the molecular layer of the cortex, which is regularly spared in an infarct, is usually disrupted at the crown of the contused gyri. In both conditions, neurons in the affected region undergo necrosis as shown by the presence of ischemic cell changes (nuclear pyknosis and cytoplasmic hypereosinophilia). However, the earliest microscopical tissue alterations include white matter edema. Approximately 3 days after the original insult, early vascular proliferation can be detected at the edge of the

Fig. 1. GBM. Tissue blocks for immunohistochemistry (Zagzag et al., 1995) and ISH (Zagzag et al., 1996) were prepared as previously described. **a.** TN-C immunoreactivity was found to be variable and heterogeneous within individual tumors. Enhanced TN-C expression was also detected among tumor cells and around individual cells as a fine fibrillary network. Occasional tumor cells showed intracytoplasmic expression of TN-C. There is strong immunoreactivity especially in hyperplastic vessels (v) including the pseudopalisading areas (p) around areas of necrosis (n). Necrotic tumor tissue remains negative. Fine fibrillar extension of TN-C from blood vessels to the surrounding tumor cells was occasionally seen. TN-C expression helped to delineate the tumor margin against the surrounding gliotic brain tissue, where it was mainly seen around hyperplastic blood vessels as previously described (Zagzag et al., 1995). Normal brain distant from the tumors showed vascular TN-C expression that was similar to the 4 samples of normal control brain i.e. TN-C was weakly expressed in the media of small intraparenchymal arterioles and leptomeningeal arteries, as previously described (Zagzag et al., 1995). Immunoperoxidase and hematoxylin counterstain, x 50. **b.** ISH for TN-C mRNA. Strong signal of TN-C mRNA is demonstrated in a hyperplastic vessel (v) and tumor cells (t) including the edge of a necrotic area (n). TN-C mRNA is seen in vascular cells lining the vascular lumens and within the walls of the vascular complexes especially at the invasive edge of the GBMs. TN-C mRNA was detected in vessels beyond the tumor "margin" in the brain tissue adjacent to the tumor in 2 out of 5. NBT/BCIP, x 50. **c.** Upregulation of TN-C mRNA expression in pseudopalisading cells (p) around necrotic areas (n). NBT/BCIP, x 50. **d.** No staining is seen with the sense probe and no detectable TN-C mRNA staining in the 4 normal brains used as controls NBT/BCIP, x 50. **e.** ISH demonstrated strong VEGF/VPF mRNA in tumor cells especially in pseudopalisading cells around areas of necrosis (n) and in areas just adjacent to the infiltrating edge of the tumors as previously described (Plate et al., 1992; Shweiki et al., 1992). NBT/BCIP, x 100. In 3 GBMs where brain tissue more distant from the tumor was present, no detectable VEGF/VPF message was found in blood vessels. ISH for VEGF/VPF was performed using a probe of the whole published sequence of VEGF/VPF (980bp). The sequence product was introduced into pBluescript II SK (Stratagene Cloning Systems, La Jolla, California). Anti-sense and sense riboprobes were prepared using digoxigenin RNA Labeling Kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). ISH for VEGF/VPF was performed using a similar protocol as for TN-C (Zagzag et al., 1996) with a few modifications. The concentration VEGF/VPF probe was 6 ng/μl. Bakers yeast was added to the hybridization buffer. Hybridization was achieved by applying 125 μl of the probe with incubation at 56 °C. Washes following hybridization were done using 2 x SSC at 56 °C and 0.2 SSC at room temperature. The alkaline phosphatase was 1:5000 dilution. Incubation with NBT/BCIP was done at room temperature in the dark. Before mounting the slides were washed with tris-EDTA and counterstained with methylene green.

necrotizing process in both infarct and trauma. By days 5 to 7 capillaries proliferate at the margin of the necrosis. Thus, in cerebral infarct edema precedes angiogenesis (Liu, 1988). Over the next 2-3 weeks neovascularization increases with marked proliferation of capillaries associated with gliosis and microglial cell activation. Hyperplastic endothelial cells with mitotic figures can be detected.

In chronic subdural hematomas (SDH), angioblastic invasion of the clot starts within a week. The new capillaries originate almost entirely from the dural aspect, (Putnam and Cushing, 1925) (i.e. from the inner dural surface). They penetrate the clot and migrate around its outer surface and then follow its inner surface. Thus, the clot becomes enclosed by a highly vascular membrane. The membrane on the dural (outer) aspect of the clot is thicker and more vascular than the inner membrane. Both membranes have formed within 2 to 3 weeks. Small blood vessels located within the capsule of the hematoma have attenuated endothelial cells and wide endothelial gap junctions (Yamashima et al., 1983) and can either "spontaneously" or after minor trauma be the source of repeated and continuing bleeding and transudation of plasma (Markwalder, 1981; Yamashima and Yamamoto, 1984). These contribute to the enlargement of the chronic SDH, rendering it a slowly expanding space-occupying lesion. Therefore,

angiogenesis within the subdural membrane plays an essential role in the organization of the chronic SDH and its enlargement.

Folkman and Klagsbrun introduced the concept of Angiogenic Diseases, and proposed to categorize as such, diseases where the dominant pathology is angiogenesis (Folkman and Klagsbrun, 1987). There is precedent for regrouping diseases with common pathological features or pathogenesis but with different etiologies. For example, inflammatory myopathies (Heffner, 1993) include dermatomyositis which is a B cell-mediated process causing vascular damage and is often associated with cancer, polymyositis which is a T cell-mediated process and inclusion body myositis, a disease of unknown etiology. Inflammatory myopathies also include infectious myopathies (e.g. trichinosis) and granulomatous myopathies (e.g. sarcoidosis). Demyelinating diseases (Prineas and McDonald, 1997) include pathological conditions of diverse etiologies. For example, multiple sclerosis has an incompletely understood etiology involving genetic and environmental factors. Adrenoleukodystrophy, known in the past as Schilder's disease, is an X-linked condition associated with an abnormal excess of very long chain fatty acid esters due to an impaired capacity to form the coenzyme A derivative. Acute disseminated encephalomyelitis follows viral infections (measles,

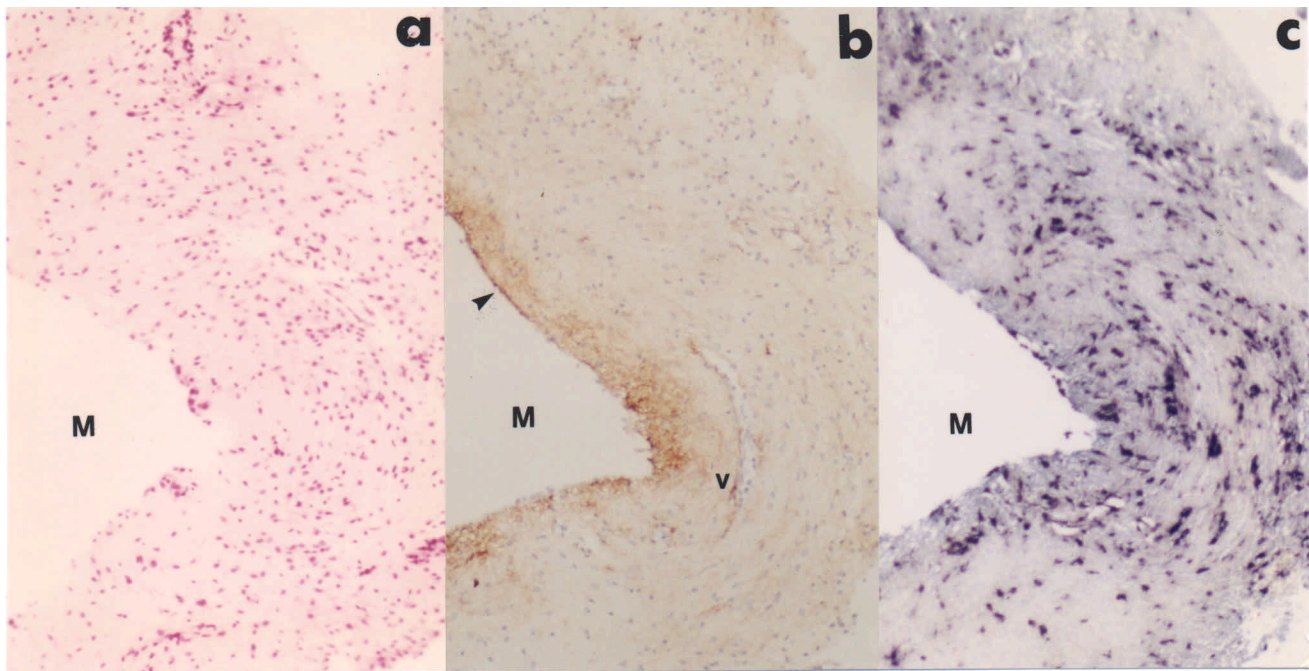


Fig. 2. Microcyst in a JPA. **a.** Moderate cellularity around a microcyst (M) in a JPA. H&E, x 100. **b.** Immunohistochemistry for TN-C of the same area as in (a) shows reactivity lining the microcyst (arrowhead) and around a vessel wall (v) adjacent to the microcyst. Immunoperoxidase and hematoxylin counterstain, x 100. TN-C was detected in hyperplastic vessels including in those lining cyst walls as previously described (Zagzag et al., 1995; Jallo et al., 1997). The immunostaining around hyperplastic vessels was either within the vascular wall or coating its outer surface. TN-C in or around vascular channels was consistently greater within and around the walls of hyperplastic vessels than non-hyperplastic blood vessels. TN-C expression was faint or focal among tumor cells but no message was detected in the tumor cells. **c.** ISH for VEGF/VPF mRNA of the same area as in (a) and (b) showing upregulation of VEGF/VPF around the microcyst. NBT/BCIP, x 100. VEGF/VPF mRNA was also detected in areas adjacent to vascular hyperplasia.

mumps, rubella, chicken pox) or vaccination (smallpox, rabies). Acute hemorrhagic leukoencephalitis (Hurst's disease) usually occurs after viral upper respiratory tract infection. Marchiafava-Bignami disease was originally described in crude red wine drinkers and is thought to be related to a vitamin deficiency. Progressive multifocal encephalopathy is due to cytopathic killing of oligodendrocytes infected with JC virus and usually occurs in immunocompromised patients. Central pontine myelinolysis is believed to be associated with the rapid correction of hyponatremia. Demyelination has also been associated with neoplasia (Peiffer, 1988). Finally, Balo's concentric sclerosis is of unknown etiology. All these conditions which have different causes are grouped together as inflammatory myopathies or demyelinating diseases because they all share a common pathological

finding, i.e. inflammation or demyelination. Similarly, the pathological conditions in the CNS in which neovascularization plays a pivotal role and where VEGF/VPF and TN-C are both upregulated could be regrouped as "Angiogenic Diseases" of the CNS.

VEGF/VPF in CNS angiogenesis

In situ hybridization (ISH) for VEGF/VPF in glioblastomas multiforme (GBMs) (Fig. 1), juvenile pilocytic astrocytomas (JPAs) (Figs. 2-4), hemangioblastomas (Fig. 5), cerebral abscesses (Fig. 6), cerebral infarcts (Fig. 7), trauma-induced cerebral lesions (Fig. 8) including 5 chronic SDHs (Fig. 9) and 4 normal control brains, demonstrates the expression of VEGF in these conditions that are associated with

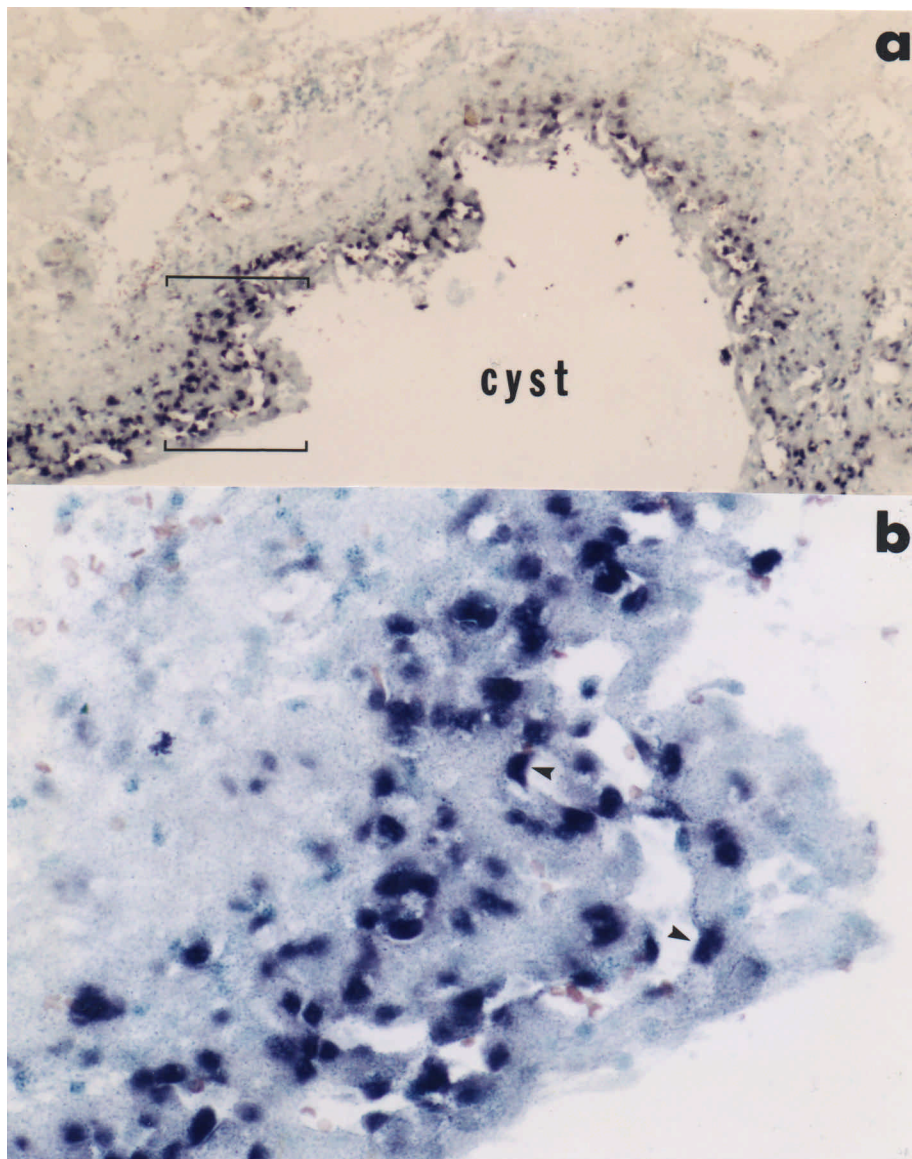


Fig. 3. ISH for VEGF/VPF in a cyst of a JPA. **a.** Wall of a large cyst showing strong signal for VEGF/VPF mRNA in the cells of hyperplastic vessels. NBT/BCIP, x 50. **b.** High magnification of the boxed area in (a). Note the strong signal for VEGF/VPF mRNA in vascular cells (arrowheads). NBT/BCIP, x 200. Only weak staining was observed in the tumor vasculature of JPAs which failed to show hyperplastic vessels. VEGF/VPF mRNA in tumor cells was focal and weak in one case and not detectable in 2 cases.

angiogenesis. Moreover, our findings demonstrate the spatial and temporal upregulation of VEGF/VPF in relation to neovascularization in both neoplastic and non-neoplastic pathological conditions of the CNS. Several lines of evidence suggest that VEGF/VPF is involved in brain tumor angiogenesis: 1) VEGF/VPF is produced by glioma cells *in vitro*; (Plate et al., 1992; Shweiki et al., 1992); 2) VEGF/VPF expression is dramatically up-regulated in various human brain tumors *in vivo*, such as highly vascularized GBMs (Plate et al., 1992; Shweiki et al., 1992), or von Hippel-Lindau disease-associated hemangioblastomas; (Stratmann et al., 1997); 3) receptors for VEGF/VPF have been demonstrated in both high and low grade gliomas (Plate et al., 1993; Weindel et al., 1994; Leung et al., 1997); and 4) experimentally induced angiogenesis and brain tumor growth in nude mice can be specifically inhibited

by anti-VEGF/VPF monoclonal antibodies (Kim et al., 1993) or by a dominant-negative flk-1 mutant (Millauer et al., 1994). Moreover, VEGF/VPF plays a role in experimental animal models of cerebral trauma (Nag et al., 1997) and infarct (Kovacs et al., 1996; Plate et al., 1999), and has been demonstrated in a variety of non-neoplastic cell types. These include neurons (Kovacs et al., 1996), astrocytes (Ijichi et al., 1995), pericytes (Murata et al., 1996), smooth muscle cells (Li et al., 1995; Stavri et al., 1995), macrophages (Berse et al., 1992), lymphoid cells (Freeman et al., 1995), platelets (Mohle et al., 1997), and fibroblasts (Volpert et al., 1997). Endothelial cells isolated from a variety of organs including skin (Namiki et al., 1995; Detmar et al., 1997), umbilical cord (Namiki et al., 1995), brain (Fischer et al., 1995), lung (Liu et al., 1995), and kidney (Seghezzi et al., 1998), *in vitro* and in organotypic cultures (Fischer

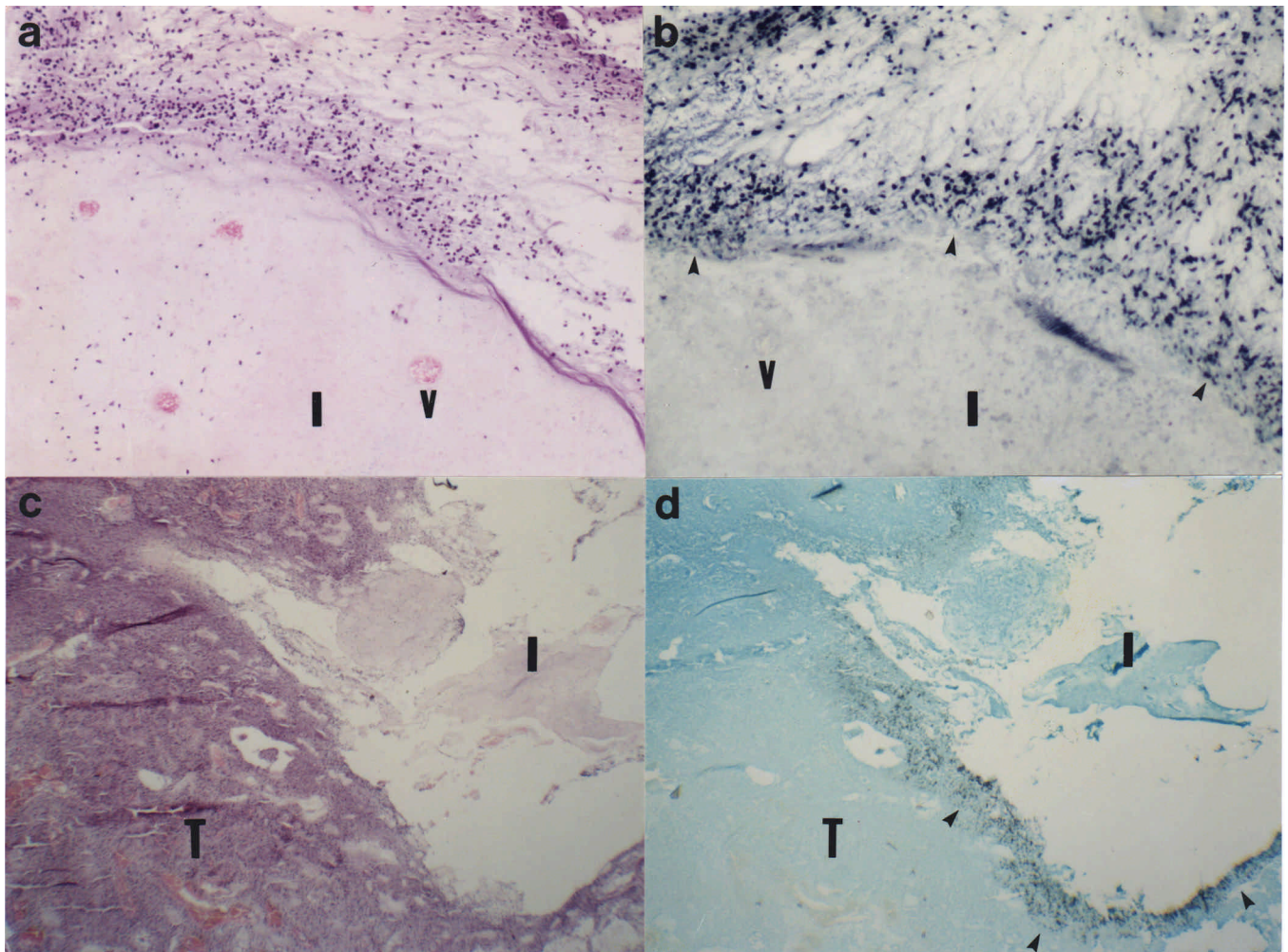


Fig. 4. ISH for VEGF/VPF mRNA in 2 JPAs with tumor infarction. High (a) and low (c) magnification of two different JPAs with tumor infarction (I). Ghost vessels can be seen in the infarcted zones (v). T: tumor adjacent to the infarct. Hx&E, a, x 100; c, x 50. High (b) and low (d) magnification of ISH for VEGF/VPF demonstrating mRNA in tumor cells around the infarcts (arrowheads). There is no VEGF/VPF in the rest of the tumor (T). NBT/BCIP, b, x 100; d, x 50. In addition in 2 out of 6 JPAs portions of the cerebellar granular layer were expressing VEGF/VPF mRNA as previously described in the normal adult rat brain (Monacci et al., 1993).

et al., 1995) have been shown to express VEGF/VPF. Our results demonstrate that under selected conditions, the role of endothelial cells in vascular growth extend beyond that of a target to involve contingency synthesis of VEGF/VPF and thus autocrine activation (Uchida et al., 1994). VEGF/VPF is angiogenic and increases

microvascular permeability (Senger et al., 1983; Roberts and Palade, 1995) including that of cerebral microvessels (Wang et al., 1996). Because cerebral angiogenesis is associated with increased vascular permeability (Zagzag et al., 1988, 1989; Del Maestro et al., 1990) which plays a major role in the pathogenesis

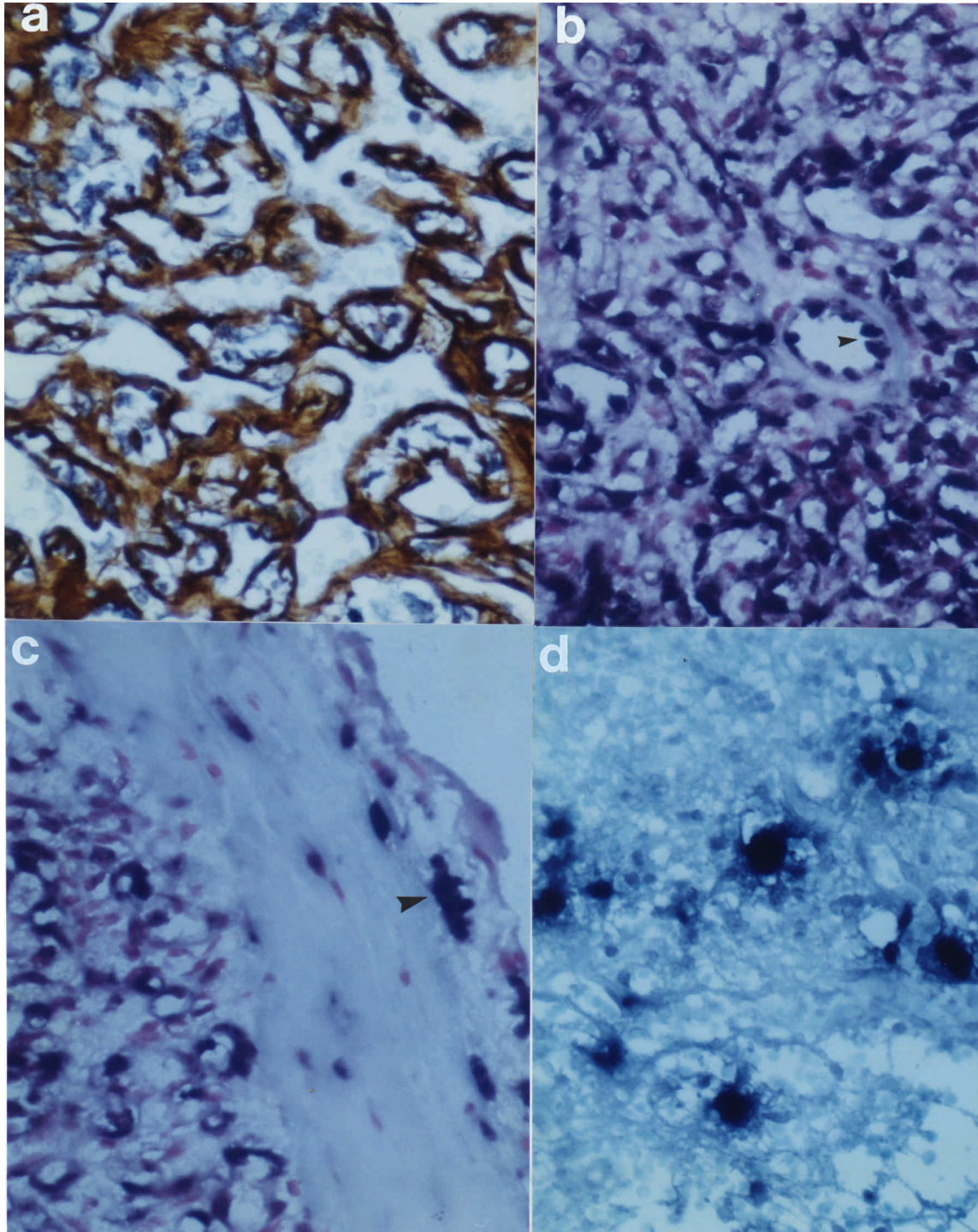
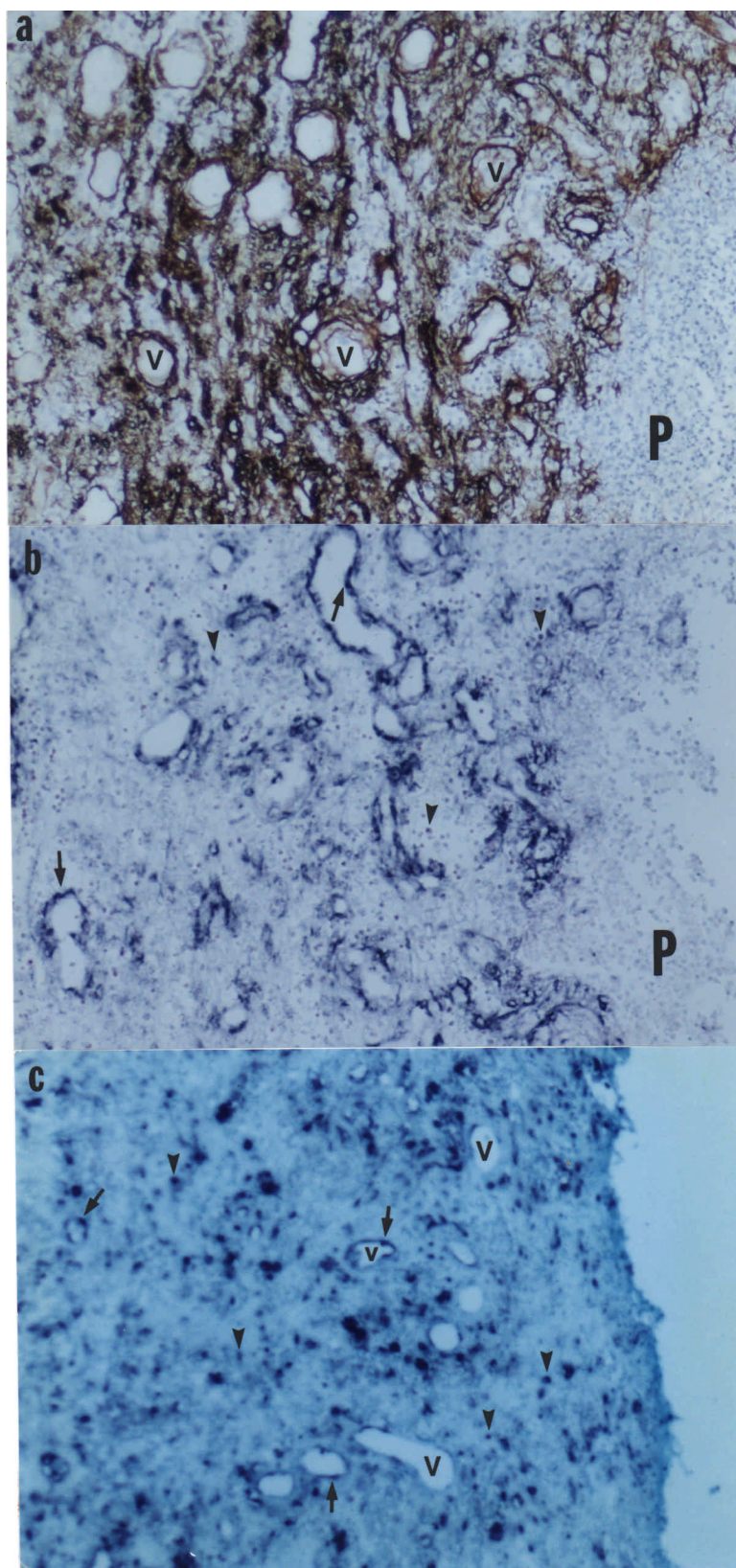


Fig. 5. Hemangioblastoma. **a.** TN-C immunostaining was heterogeneous and found around vascular channels, often as a fine network radiating out from larger size vessels. Immunoperoxidase and hematoxylin counterstain, x 100. **b.** TN-C mRNA signal is intense in vascular cells of the tumor including endothelial cells of capillaries (arrowheads). NBT/BCIP, x 200. **c.** Some vascular cells within the wall of larger vessels are also labeled for TN-C mRNA (arrowhead). NBT/BCIP, x 400. **d.** Strong VEGF/VPF mRNA is detected in stromal cells. NBT/BCIP, x 400. As previously described (Stratmann et al., 1997).



of cerebral edema (Del Maestro et al., 1990; Hariri, 1994), it is likely that the development of cerebral edema is intimately linked to angiogenesis. Moreover, in several conditions, edema formation precedes angiogenesis, e.g. in cerebral infarct (Liu, 1988). Our study suggests that VEGF/VPF could play an important role in the pathogenesis of angiogenesis associated-cerebral edema in the CNS which interestingly has been referred to as vasogenic edema (Klatzo, 1967). Ultrastructural cellular changes seen in cells exposed to VEGF/VPF include fenestrations (Roberts and Palade, 1995) and activated vesiculo-vacuolar organelles (Feng et al., 1996). Fenestrations (Long, 1970) and vesiculo-vacuolar organelles (Lossinsky et al., 1996) have been described in brain tumours vessels. Moreover, vesiculo-vacuolar organelles (Lossinsky et al., 1996) and capillary fenestrations have been linked to barrier permeability in brain tumors (Long, 1970) and in SDHs (Yamashima et al., 1983). It is interesting that among the 3 types of neoplasms we have studied, JPAs and hemangioblastomas which are often cystic are usually less likely to be associated with peritumoral edema. This suggests that the storage of VEGF/VPF primarily occurs in the tumor associated cyst (Weindel et al., 1994), rather than in the surrounding brain tissue as it probably occurs in most GBMs. In our study when VEGF/VPF mRNA was found just beyond the infiltrating edge of the GBMs.

Cerebral edema is one of the most important factors contributing to the morbidity and mortality associated with these edematogenic CNS diseases that we have

Fig. 6. Organizing wall of a cerebral abscess. **a.** Strong immunoreactivity for TN-C is seen around proliferating vascular channels (V). It was diffusely seen around vessels, delineating vascular channels by following the branches of the arborization pattern, it was also detected in the intervascular stroma and in the subendothelial matrix forming a thick band at the interphase between endothelium and stroma. Immunoperoxidase and hematoxylin counterstain, x 50. Necrotic tissue and areas composed mainly of neutrophils (p) were negative for TN-C immunostaining. **b.** ISH for TN-C mRNA shows signal within vascular cells (arrows), fibroblasts and inflammatory cells (arrowheads). Note lack of TN-C immunoreactivity and TN-C mRNA in necrotic regions within the purulent exudate (P). Perivascular staining was stronger than the intervascular staining and was diffusely expressed in the extracellular space as a discrete fibrillary network surrounding individual or groups of inflammatory cells; TN-C mRNA was also seen in reactive astrocytes, in brain tissue adjacent to the abscess. NBT/BCIP x 50. **c.** ISH for VEGF/VPF shows many inflammatory, fibroblastic and astrocytic cells (arrowheads) expressing VEGF/VPF in between vessels (v) some with labeled endothelial cells (arrows). VEGF/VPF mRNA. NBT/BCIP, x 50

studied. For example, it often complicates the post operative period of patients with brain tumors (Hariri, 1994). In cerebral abscesses, edema is often widespread (Klatzo, 1967; Nakagawa et al., 1990) and develops

early, greatly increasing the mass effect of the local lesion. It is the major cause of early death in cerebral infarcts (White et al., 1979; Ropper and Shafren, 1984). In cerebral traumas, (Bruce et al., 1981) cerebral edema

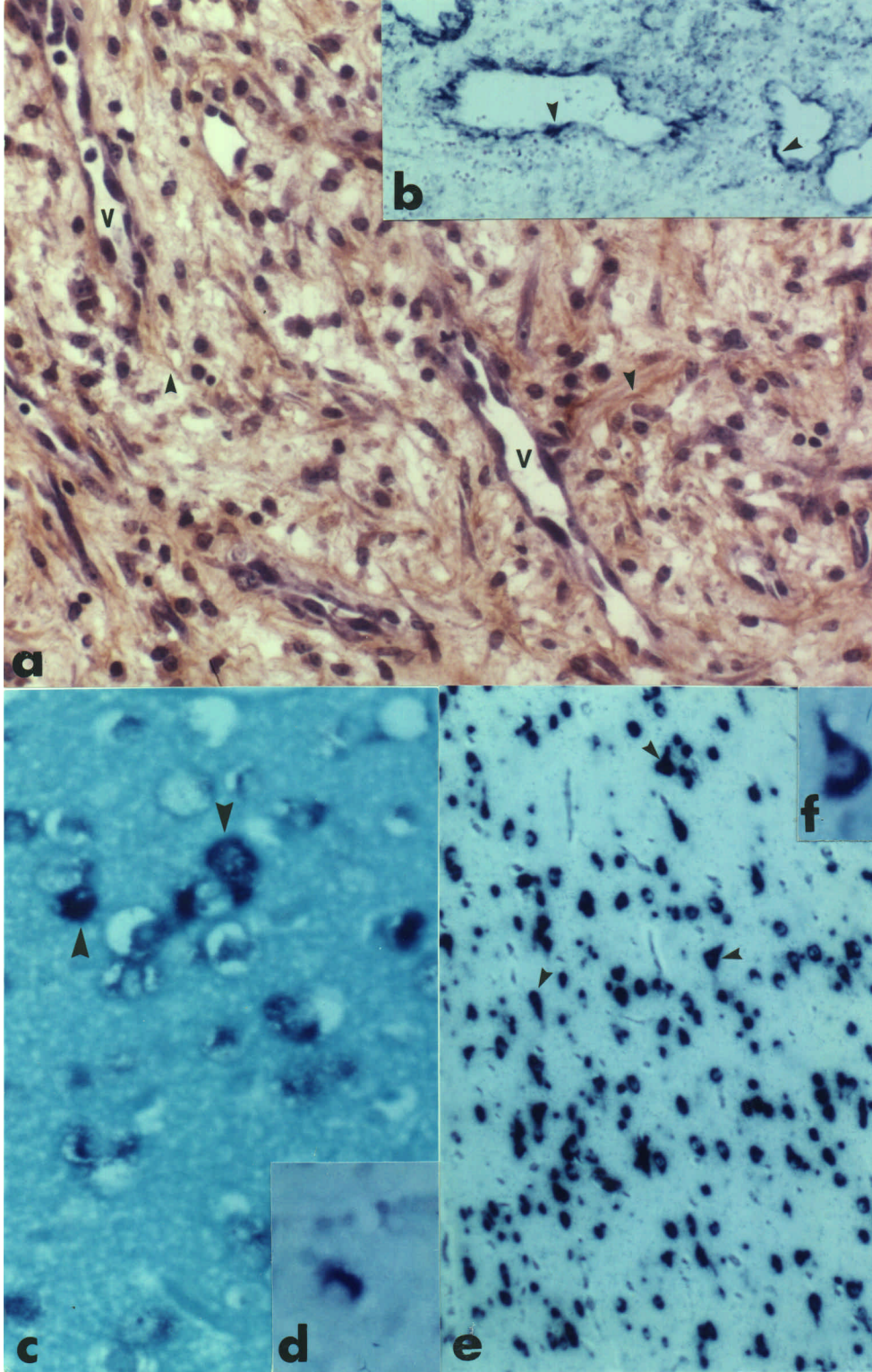


Fig. 7. Cerebral infarct. **a.** Fibrillary TN-C immunostaining (arrowheads) at the edge of an infarct where numerous new blood vessels are present (v). TN-C expression was minimal or undetectable in the center of the infarct, with enhanced fibrillary staining at the periphery of the lesion primarily around vascular channels and was more abundant in areas where lumens were discernible and plump endothelial cells were seen. TN-C mRNA was primarily seen in capillaries and in reactive astrocytes. Immunoperoxidase and hematoxylin counterstain, x 100. **b.** ISH for TN-C shows strong signal within vascular cells (arrowheads). NBT/BCIP, x 50. **c.** ISH for VEGF/VPF shows signal within macrophages (arrowheads) and vascular cells. NBT/BCIP, x 200. **d.** Irregular nuclei resembling microglial cells were also labeled. **e.** Cortex adjacent to an infarcted area of brain tissue showing upregulation of VEGF/VPF within neurons (arrowheads). NBT/BCIP; x 50. **f.** Higher magnification of a pyramidal neuron labeled for VEGF/VPF mRNA. NBT/BCIP, x 200.

is variable. However, even in a patient with a small cerebral contusion, edema may involve the majority of white matter of the hemisphere bearing a focal injury. In all these processes it adds to the increased intracranial pressure caused by the primary lesion by superimposing a significantly larger mass on the brain. It may worsen the neurological condition with the development of

hemiparesis, speech dysfunction, and convulsions, and in more severe cases, brain swelling may cause a fatal cerebral herniation syndrome with secondary damage to the brainstem. Cerebral edema is therefore a key component in determining prognosis and clinical outcome. The potentially lethal aspect of cerebral edema is especially well illustrated by the significant decline in

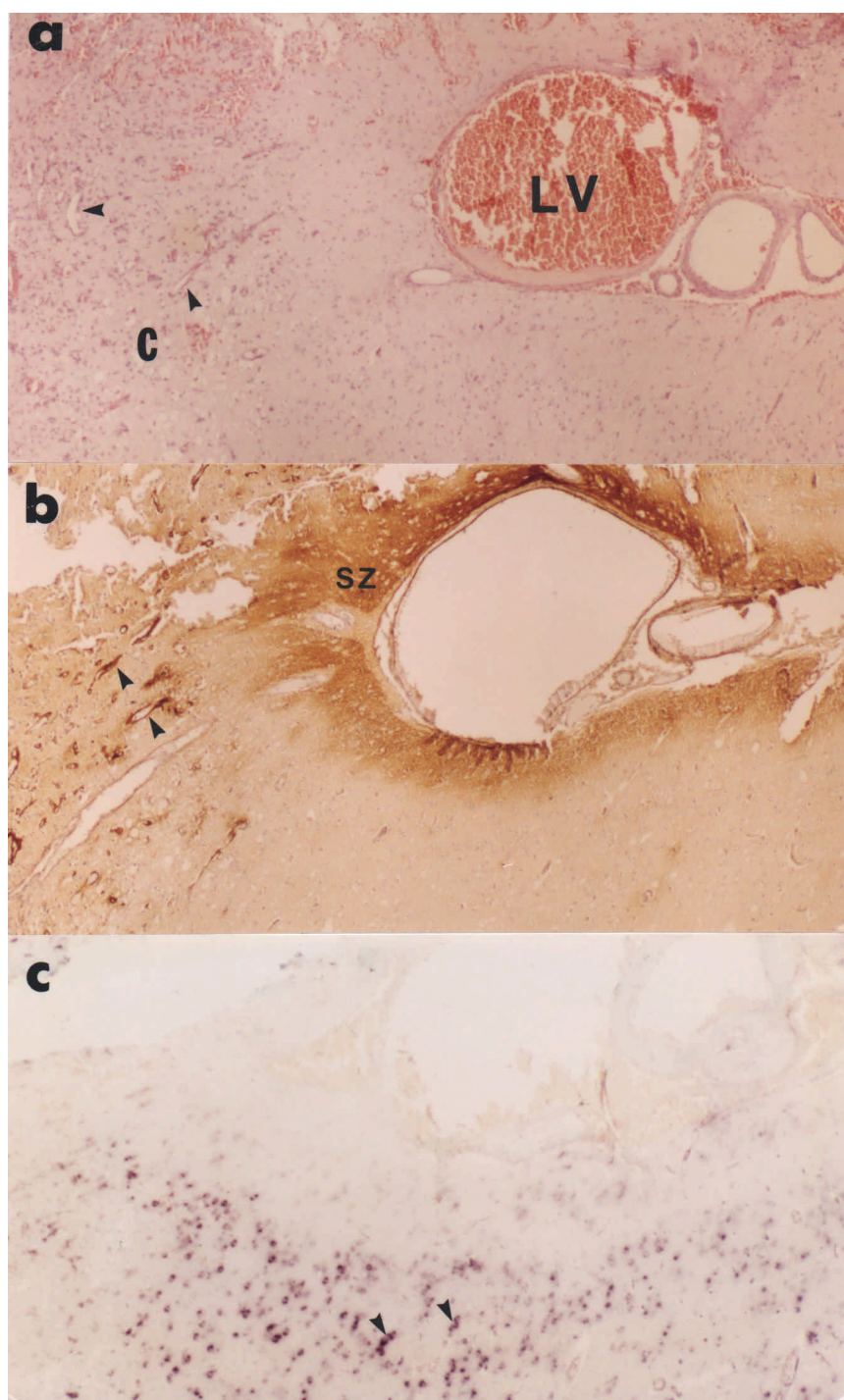


Fig. 8. Cerebral contusion. **a.** Histological section of cortex and subarachnoid space with distended leptomenigeal vessel (LV) at the edge of a contused area (c) showing many blood vessels (arrowheads). H&E, x 50. **b.** Immunohistochemistry for TN-C of the same area described in (a). Note the strong reactivity around vessel (arrowheads) in the contused region and in the subpial zone (SZ). In blood vessels with flat endothelial cells and areas without obvious vascular lumens, TN-C expression was weak. Intracellular TN-C was seen in rare reactive astrocytes. Immunoperoxidase and hematoxylin counterstain, x 50. **c.** ISH for VEGF/VPF mRNA of the same area described in (a) and (b). Upregulation for VEGF/VPF is seen adjacent to the contused area primarily in neurons (arrowhead). Macrophages and vascular cells are also labeled. NBT/BCIP; x 50

neurosurgical operative mortality and by the dramatic neurological improvement in non-surgical patients associated with the use of corticosteroid antiedematous therapy (Jelsma and Bucy, 1967) shown to downregulate VEGF/VPF expression *in vitro* (Criscuolo et al., 1988). Despite progress in understanding the nature, pathophysiology and therapy of cerebral edema, still, it remains a common and ongoing problem for many patients with brain pathology.

VEGF/VPF expression is modulated by hypoxia, glucose deficiency, tumor suppressor genes, oncogenes and other cytokines (Claffey and Robinson, 1996). The CNS tumoral or reparative processes that we have studied are likely to be associated with some degree of hypoxia. Astrocytomas of both low (Giannini and Scheithauer, 1997) and high grade (Barker et al., 1996) display necrotic and/or infarcted areas. Abscesses have necrotic centers and thus have ischemic/hypoxic tissues. Infarcts by their nature have an obvious ischemic component. Besides infarction that can be identified in 90% of fatal head injuries (Graham et al., 1995), vasospasm due to subarachnoid hemorrhage with cerebral contusions can contribute to the developing brain edema by induction of ischemia. Moreover,

cerebral contusions are usually associated with full thickness necrosis of the cortex. In both, the signal in elongated cells is consistent with microglial cells (Barleon et al., 1996), where a VEGF/VPF signal was detected. VEGF/VPF is expressed by cells in the deep portion of the subdural membrane. It is interesting that these cells expressing VEGF/VPF also express CD31 (PECAM-1) that has been recently shown to be implicated in angiogenesis (DeLisser et al., 1997). VEGF/VPF may represent the angiogenic factor that has been extracted from SDH (Nakamura and Tsubokawa, 1989). VEGF/VPF upregulation is detected as quickly as 3 hours *in vitro* in astrocytes exposed to hypoxia (Ijichi et al., 1995), and within 30 minutes in polymorphonuclear neutrophils after injury *in vivo* (Nag et al., 1997). Thus, hypoxia is critical for the upregulation of VEGF/VPF. HIF-1 α plays an essential role in oxygen homeostasis (Wang and Semenza, 1995; Wang et al., 1995; Iyer et al., 1998). HIF-1 activates a large battery of genes whose protein products include VEGF/VPF (Forsythe et al., 1996; Semenza et al., 1996; Iyer et al., 1998; Semenza, 1998; Tazuke et al., 1998; Feldser et al., 1999). HIF-1 has been demonstrated in many tumors (Zagzag et al., 2000). However, in some

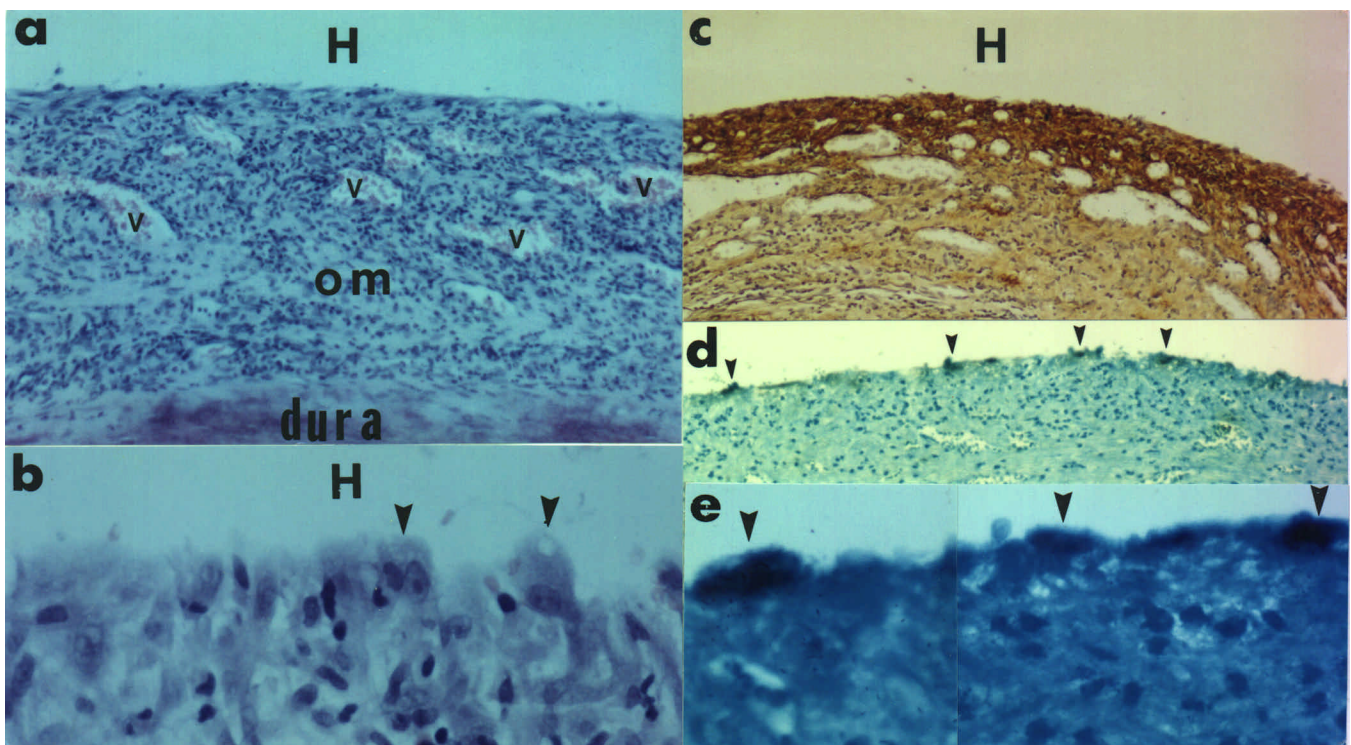


Fig. 9. Chronic SDH. **a.** Outer membrane (OM) adjacent to the dura mater lining the hematoma cavity (H) showing the presence of an inflammatory infiltrate and numerous newly formed vascular channels (V). H&E, x 50. **b.** Higher magnification of the inner portion of the membrane shows large cells (arrowheads) with large nuclei and prominent nucleoli lining the hematoma cavity (H). These cells were also immunopositive for vimentin, smooth muscle actin, CD31 (PECAM-1) and negative for Factor VIII related antigen and CD34 (Q-BEND10) (data not shown). Smaller mononuclear cells are located deeper in the membrane. H&E, x 400. **c.** TN-C is heterogeneously expressed in the outer membrane. TN-C immunostaining is primarily seen in the areas adjacent to the hematoma cavity (H) i.e. in the inner deeper layer of the outer membrane where it is marked around vascular channels. Immunoperoxidase and hematoxylin counterstain, x 50. **d.** ISH for VEGF/VPF mRNA shows upregulation within the large cells (arrowheads) lining the hematoma cavity in the deeper portion of the outer membrane. NBT/BCIP, x 50. **e.** Higher magnification of these cells (arrowheads). NBT/BCIP, x 400

conditions, e.g. hemangioblastomas, other mechanisms (i.e. loss of the Von Hippel-Lindau tumor suppressor gene (Siemeister et al., 1996; Stratmann et al., 1997; Vortmeyer et al., 1997; Lee et al., 1998), are responsible for the upregulation of VEGF/VPF. Other tumor suppressor genes and oncogenes including ras (Rak et al., 1995), src (Mukhopadhyay et al., 1995; Jiang et al., 1997), and p53 (Kieser et al., 1994; Mukhopadhyay et al., 1995) have been shown to modulate VEGF/VPF expression. Both src (Kieser et al., 1994; Mukhopadhyay et al., 1995), which at least in part involve HIF-1 upregulation (Jiang et al., 1997), and ras (Rak et al., 1995) upregulate VEGF/VPF. The exact implication of p53 is unclear. While mutant p53 was reported to potentiate the upregulation of VEGF/VPF by protein kinase C (Keiser et al., 1994), other studies have demonstrated that wild type p53 suppresses the src-induced VEGF/VPF transcription (Mukhopadhyay et al., 1995). Some studies have implicated the p53-MDM2 pathway in the regulation of HIF-1 degradation (Ravi et al., 2000). Epidermal growth factor (EGF) receptor upregulates VEGF/VPF (Petit et al., 1997). Both P53 and EGFR are important in the pathogenesis of high grade gliomas (Louis and Gusella, 1995). The role of src and ras in human brain tumors remains to be clarified (Wasson et al., 1990; Brustle et al., 1992; Patt et al., 1993). Glucose deficiency is also able to induce VEGF/VPF expression (Shweiki et al., 1995).

Direct [basic fibroblast growth factor (FGF) (Stavri et al., 1995; Tsai et al., 1995; Ryuto et al., 1996), platelet derived growth factor (PDGF) (Finkenzeller et al., 1992; Tsai et al., 1995), EGF (Tsai et al., 1995)] and indirect [tumor necrosis factor (TNF) (Ryuto et al., 1996; Yoshida et al., 1997), transforming growth factor (TGF) (Pertovaara et al., 1994) and interleukin-1 (Li et al., 1995; Ryuto et al., 1996)] angiogenic factors also upregulate VEGF/VPF expression. Some have synergistic effects with VEGF/VPF, e.g. basic FGF (Goto et al., 1993). Moreover, FGF-induced angiogenesis, is at least in part, mediated by VEGF/VPF (Deroanne et al., 1997). Thus, a variety of potentially interrelated pathways can lead to the upregulation of VEGF/VPF. These modulating factors, including hypoxia, glucose deficiency, tumor suppressor genes, oncogenes and growth factors, probably interact *in vivo* in a complex interplay. For example, hypoxia modulates p53 expression (Graeber et al., 1994) and is linked to permeability (Tanno et al., 1992), probably in part through upregulation of VEGF/VPF. Thus, each of these regulating mechanisms alone or in conjunction with others may lead to the upregulation of VEGF/VPF. Further studies are needed to elucidate their interaction. High (Plate et al., 1992; Shweiki et al., 1992) and low (Weindel et al., 1994; Leung et al., 1997) grade human astrocytomas, and hemangioblastomas (Stratmann et al., 1997) and experimental animal models of CNS neoplasms (Plate et al., 1993), cold injury (Nag et al., 1997) and ischemia (Kovacs et al., 1996), are associated with VEGF/VPF upregulation. We have demonstrated

that a variety of human hypoxic/ischemic, inflammatory, infectious and traumatic conditions of the CNS associated with angiogenesis are also associated with upregulation of VEGF/VPF, a potent angiogenic and edematogenic cytokine.

TN-C and angiogenesis

In contrast to the low levels of TN-C found in normal adult brain, enhanced expression occurs in human astrocytomas (Zagzag et al., 1995, 1996). For example, by Western blot its expression is elevated up to 4 fold in GBMs as compared to normal control tissue (Zagzag et al., 1995). It is expressed around tumor cells mainly of high-grade tumors as well as in hyperplastic vessels of astrocytomas regardless of grade, and its expression correlates with angiogenesis (Zagzag et al., 1995, 1996). Immunohistochemistry and ISH for TN-C showed enhanced TN-C expression in all high and low-grade astrocytomas (Figs. 1, 2) and hemangioblastomas (Fig. 5), and in a variety of non-neoplastic diseases of the CNS which are associated with neovascularization. These included infectious, inflammatory and ischemic diseases of the CNS, e.g. cerebral abscesses (Fig. 6) and infarcts (Fig. 7), as well as traumatic conditions such as cerebral contusions (Fig. 8) and SDHs (Fig. 9). TN-C was observed around hyperplastic blood vessels of tumors regardless of their grade or type as well as around newly formed vascular channels of non-neoplastic processes. Thus, TN-C expression correlates spatially and temporally with angiogenesis in both neoplastic and non-neoplastic human diseases of the brain. Moreover, because TN-C mRNA and protein were not upregulated in vessels of normal brain, it is possible that TN-C expression might be important for vascular cell activation. Vascular cells able to synthesize TN-C include endothelial cells (Webersinke et al., 1992; Hahn et al., 1995; Zagzag et al., 1996), and pericytes/smooth muscle cells (Schor et al., 1991; Mackie et al., 1992; Zagzag et al., 1996). Other cell types capable of expressing TN-C include astrocytes (Grumet et al., 1985; Dorries et al., 1993; Brodkey et al., 1995; Zagzag et al., 1996; Ness and David, 1997), fibroblasts (Copertino et al., 1997), and neurons (Ferhat et al., 1996).

Evidence linking TN-C and angiogenesis includes: 1) TN-C, which has both adhesive and counteradhesive domains for a variety of cell types (Prieto et al., 1992) modulates endothelial cell adhesion (Murphy-Ullrich et al., 1991; Joshi et al., 1993; Sriramarao et al., 1993; Chung and Erickson, 1994). This is mediated in part by $\alpha_3\beta_3$ integrin (Joshi et al., 1993; Sriramarao et al., 1993) that is required for angiogenesis (Brooks et al., 1994). 2) TN-C modulates microvascular migration (Kaplon et al., 1991; Canfield and Schor, 1995; Hahn et al., 1995; Chung et al., 1996). For example, TN-C-rich matrices are permissive for endothelial cell migration, by contrast to inhibitory thrombospondin-rich matrices (Canfield and Schor, 1995). TN-C is specifically expressed at the

site of migration of developing embryonic vasculature (Spence and Poole, 1994). Moreover, during cornea development, cells derived from the neural crest and destined to become endothelia migrate exactly along the line of the TN-C-rich stroma (Kaplony et al., 1991). Furthermore, antibodies against TN-C inhibit endothelial cell sprouting *in vitro* (Canfield and Schor, 1995; Hahn et al., 1995; Chung et al., 1996). TN-C also modulates the migration of glial (Wehrle-Haller and Chiquet, 1993) and glioma cells (Deryugina and Bourdon, 1996), cerebellar granular layer cells (Husmann et al., 1992) and supports lymphocyte rolling (Clark et al., 1997). TN-C also plays a similar role during embryogenesis (Erickson and Bourdon, 1989). For example, TN-C is expressed at the site of migration of neural crest cells (Mackie et al., 1988), which could be inhibited by anti-TN-C antibodies (Bronner-Fraser, 1988). 3) TN-C modulates migration and proliferation of vascular cells, both crucial steps of the angiogenic cascade (Ausprunk and Folkman, 1977). TN-C modulates the proliferation of a variety of cell types (Chiquet-Ehrismann et al., 1986; Crossin, 1991). However, the mitogenic effect of TN-C on endothelial cells is seen only if TN-C is added before or simultaneously when bFGF is added to endothelial cell cultures (Chung et al., 1996). TN-C enhances cell migration both by its anti-adhesive effects (Murphy-Ullrich et al., 1991; Joshi et al., 1993; Sriramarao et al., 1993; Chung and Erickson, 1994) and by stimulation of the expression of genes encoding matrix metalloproteinases (Tremble et al., 1994). One additional important mechanism is the loss of focal adhesion in endothelial cells induced by the alternatively spliced region of TN-C that is a step associated with cell migration and proliferation (Murphy-Ullrich et al., 1991; Chung and Erickson, 1994). This effect can be blocked by antibodies against annexin II, a 35 kD non-integrin receptor for TN-C on endothelial cells (Murphy-Ullrich et al., 1991; Chung and Erickson, 1994). Interestingly, overexpression of an immediate early gene, e.g. c-jun which is upregulated in brain infarcts (Liu, 1995) and injuries (Nag et al., 1997) and associated with angiogenesis (Michel et al., 1994; Liu, 1995; Nag et al., 1997) stimulate TN-C (Mettouchi et al., 1997). Recently TN-C has been shown to be a survival factor for vascular smooth muscle cells (Jones et al., 1997), which have been implicated in the neovascular proliferative phenomena associated with GBMs (Haddad et al., 1992); 4) TN-C is up-regulated spatially and temporally in newly formed vessels of granulation tissue in experimentally induced skin wounds (Mackie et al., 1988; Chuong and Chen, 1991) and is not detectable or markedly reduced in the scar when wound contraction is complete (Chuong and Chen, 1991; Fassler et al., 1996). 5) TN-C is expressed in vascular tumors and reactive vascular proliferations e.g. bacillary angiomatosis (Kostinansky et al., 1997). 6) TN-C binds to heparin, (Weber et al., 1995) an important modulator of angiogenesis (Folkman and Shing, 1992). 7) vascular cells including endothelial cells and smooth muscle

cells/pericytes contribute to the deposition of TN-C (Webersinke et al., 1992; Hahn et al., 1995; Zagzag et al., 1996) present at sites of vascular hyperplasia. 8) several factors known to stimulate angiogenesis in cerebral embryogenesis and neoplasia (Zagzag, 1995), including basic FGF (Rettig and Garin-Chesa, 1989; Meiners et al., 1993; Tucker et al., 1993; Rettig et al., 1994), TGF (Rettig and Garin-Chesa, 1989; Adams Pearson et al., 1988; Mackie et al., 1992; Hahn et al., 1995), PDGF (Adams Pearson et al., 1988; Mackie et al., 1992), EGF (Sakai et al., 1995), interleukin-1 (Rettig et al., 1994), and TNF-alpha (Rettig and Garin-Chesa, 1989; Rettig et al., 1994) can upregulate TN-C expression.

Although a variety of ECM molecules including laminin (Kubota et al., 1988), fibronectin (Nicosia et al., 1993), collagen (Montesano et al., 1983), thrombospondin (Iruela-Arispe et al., 1991), SPARC (Lane et al., 1994), and vitronectin (Davis et al., 1993) have been implicated in the regulation of angiogenesis, it appears that the interaction of endothelial cells with TN-C is different from that of the other ECM molecules. For example, endothelial cells *in vitro* attach to TN-C substrata where they elongate and extend and have interconnecting processes (Sriramarao et al., 1993). These features are lacking when endothelial cells are grown on fibronectin, collagen, vitronectin or laminin substrata (Sriramarao et al., 1993). Because of its particular implication in brain pathology, and its potential role in each of the crucial steps of the angiogenic cascade, TN-C may prove to be the most important ECM molecule in CNS pathological angiogenesis.

Conclusion

The strong association of increased VEGF/VPF and TN-C expression in angiogenic conditions of the CNS suggests a link between their expression. Whether VEGF/VPF upregulates TN-C expression or how TN-C precisely modulates angiogenesis is unknown. The effect of VEGF/VPF, a hypoxia-inducible angiogenic factor, on TN expression is unknown. Since TN-C lacks a hypoxia response element, the upregulation of TN in a hypoxic environment, could be mediated by VEGF/VPF. Thus, it is of interest to investigate if VEGF/VPF upregulates TN expression. TN-C may be an angiogenic cofactor by presenting VEGF/VPF to the cell surface as it was described for proteoglycans and FGF (Schlessinger et al., 1995). Besides VEGF/VPF and TN-C, there are other molecules which are also upregulated in a variety of neoplastic and non-neoplastic conditions of the CNS associated with angiogenesis. For example, $\alpha_3\beta_3$ integrin required for angiogenesis (Brooks et al., 1994), is upregulated in embryogenesis (Sutherland et al., 1993), in brain neoplasms (Gladson, 1996) and also in cerebral ischemia (Okada et al., 1996). VEGF/VPF and TN-C follow the same paradigm of upregulation in embryogenesis, become almost undetectable in adult

quiescency, and are re-upregulated in tissue injury and activated state. It is therefore likely that embryological, neoplastic and non-neoplastic angiogenesis in the brain is mediated by similar biological compounds and molecules. The accurate regulation of the well-controlled angiogenesis occurring in embryogenesis as opposed to the uncontrolled neovascularization of tumors remains unclear. Nevertheless, VEGF/VPF and TN-C are upregulated in several human pathological neoplastic and non-neoplastic processes of the CNS associated with angiogenesis.

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