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ORIGINAL ARTICLE

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Course of glial immunoreactivity for vimentin, tenascin and α1-antichymotrypsin after traumatic injury to human brain

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Abstract In a total of 104 individuals who had sustained traumatic brain injury (TBI), the course of glial immunoreactivity was investigated at the injured cortical area during the first 30 weeks after the trauma, in order to provide data for a forensic wound age estimation. Glial cells were stained with antibodies against the intermediate filament protein vimentin, the extracellular matrix protein tenascin and the serine protease inhibitor α1-antichymotrypsin (α1-ACT). Injury-induced glial staining reactions could be observed, at the earliest, after a post-infliction interval of 3.1 h for α1-ACT, 22 h for vimentin and 7 days for tenascin.

Keywords Human brain injury · Glial reaction · Immunohistochemistry · Wound age

Introduction

Reactive gliosis is a common phenomenon in the central nervous system (CNS) following tissue destruction induced by degenerative diseases or by trauma [19]. It is characterised by astrocyte proliferation and extensive hypertrophy of the cell body and cytoplasm [15], accompanied by angiogenesis and deposition of a dense fibrous glial/meningeal scar at the lesion site [23]. Although reactive gliosis has long been considered the major impediment to axonal regrowth [4, 13, 18, 28], the formation of a glial barrier around a lesion site can also isolate the still intact CNS tissue from secondary lesions [8].

In addition, some new data strongly suggest that under certain conditions reactive astrocytes could provide a permissive substratum for neuritic extension [29].

Over the past decade, the development of numerous antibodies which recognise different surface molecules on reactive astrocytes as well as growth factors, cytokines or enzymes has advanced our knowledge of neuroglial interactions occurring during development and after various types of CNS injury. For morphological identification of reactive astrocytes in the intact and lesioned CNS, the intermediate filament proteins GFAP and vimentin have been the markers most often used in previous studies [29]. Furthermore, it could be demonstrated that immunohistochemical investigations on the GFAP expression following human brain injury are of considerable interest for forensic wound age estimation [18]. In order to obtain further information on the course of traumatically induced glial immunoreactivity, we investigated the time-dependent glial expression of vimentin, tenascin and α1-antichymotrypsin during the first 30 weeks after brain injury in humans.

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Material and methods

Brain tissue with macroscopically visible cortical contusions was obtained at autopsy from 104 individuals aged between 6 and 81 years (average age 44 years) who had sustained closed head injury. The survival period ranged between a few minutes and 30 weeks, and the post-mortem interval did not exceed 3 days. All individuals with a survival period up to 3 weeks died from cerebral dysregulation caused by neuronal damage, while in the remaining cases (n = 4) secondary complications such as pneumonia (n = 2) or embolism of the lung (n = 1) were found. One patient died from acute coronary insufficiency. Neither secondary haemorrhages, disturbances of blood coagulation which might influence the wound healing processes, nor previous CNS pathologies were evident according to the clinical data.

Cortical samples of macroscopically unaltered cerebral regions as well as brain tissue from 32 individuals without head injury who died of acute cardiac arrest (n = 17), traumatic asphyxia (n = 10) or embolism of the lung (n = 5) served as controls. The individual age of the control group ranged between 8 and 78 years, mean 47 years.

After fixation in 4% PBS-formaldehyde solution for a maximum of 24 h the tissue samples were embedded in paraffin and sections (3–5 μm) were stained with hematoxylin and eosin (H & E). Specimens with signs of autolytic changes such as post-mortem cell shrinkage or diminished nuclear stainability in H & E stained preparations as well as specimens showing pathological changes were excluded.

Immunohistochemistry

The following antigens were detected using the avidin-biotin-complex (ABC) method according to the manufacturer's recommended protocols:

1. Vimentin (Linaris, E 024): monoclonal vim 3B4, no dilution, pretreatment with pronase
2. Tenascin (DAKO, M 0636): monoclonal TN2, dilution 1:25, pretreatment with pronase
3. α1-ACT (DAKO, A 0022): polyclonal, dilution 1:100, no pretreatment

Results

Vimentin

Glial cells showed no positive vimentin reaction in uninjured brain tissue from individuals who had sustained blunt force brain injury or from the control group, whereas the mesenchymal structures in blood vessels and in the pia mater were regularly positive for vimentin.

In contrast, a distinct cellular vimentin expression was found in damaged brain tissue from individuals who had sustained traumatic brain injury. A positive staining reaction was visible in a cortical contusion with a survival time of 22 h at the earliest, and 7 out of 28 cases with a survival time between 22 h and 6 days were positive for vimentin. The vimentin-positive cells were characterised by large, round-shaped cell bodies, cytologically equal to microglia cells (Fig. 1 A). In cortical lesions with a wound age of at least 6 days and up to 4 weeks, in all cases (n = 18) high numbers of vimentin-positive glial cells were found adjacent to the lesion site. In addition to these microglia-like cells, astrocytes with numerous fibrous processes could be stained in cases with this survival interval (Fig. 1 B).

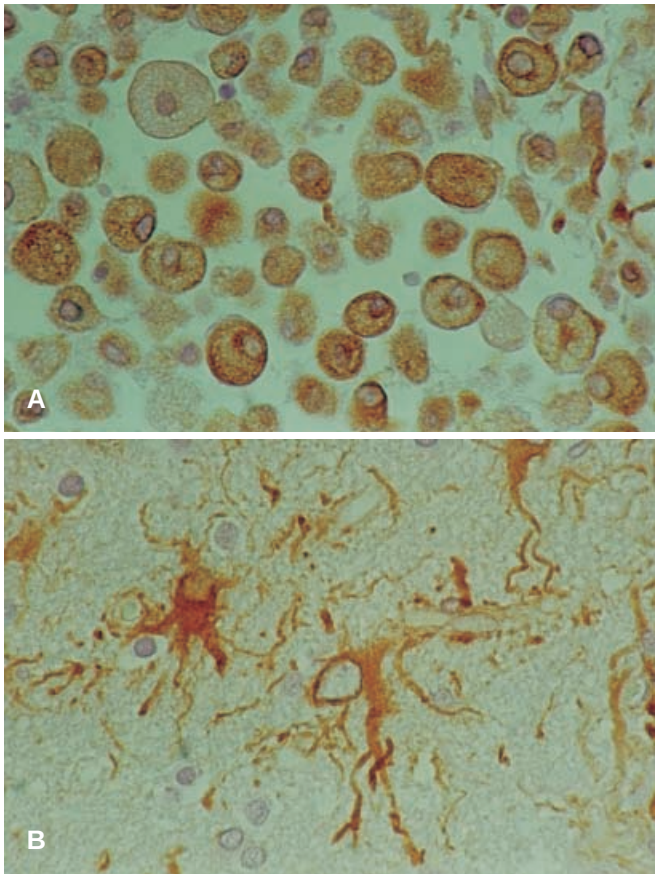

Fig. 1 A, B Glial expression of vimentin. Large round-shaped cell bodies at the lesion site positive for vimentin, **A** Seven days after the injury (× 410). **B** Vimentin-positive glial cells with numerous fibrous processes in a cortical contusion with a wound age of 14 days (× 410)

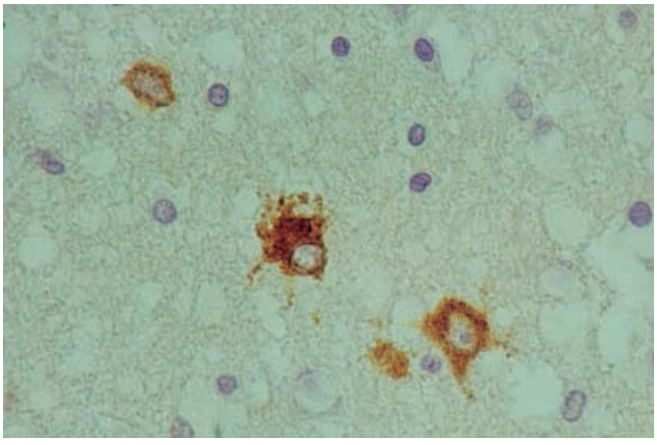

Fig. 2 Glial expression of tenascin in a cortical contusion 8 days after the injury (× 410)

Tenascin

In uninjured brain tissue no tenascin immunoreactivity was found, either in glial cells or in the vasculature or meninges, whereas in cortical contusions a positive glial staining could be observed during a certain phase of wound healing. Distinct tenascin expression by glial cells adjacent to the damaged area was detectable in a cortical lesion with a wound age of 7 days at the earliest, and up to 14 days after trauma the majority of cases (about 75%) were positive (Figs. 2 and 3). In cortical contusions older than 4 weeks, a positive tenascin reaction could not be detected.

α1-antichymotrypsin

Using the antibody against α1-ACT, 6 out of 30 control cases as well as 11 out of 104 cases with traumatically injured brain tissue were excluded from the examination due to significant background staining. The remaining 24 cases with uninjured brain tissue showed no cellular α1-ACT immunoreactivity. In contrast, distinct α1-ACT expression could be observed following brain injury (Fig. 4). A positive staining reaction was visible in a cortical contusion with a wound age of 3.1 hours at the earliest, and 25 out of 36 cases (approx. 69%) with a post-infliction interval between 1 and 13 days showed α1-ACT-positive

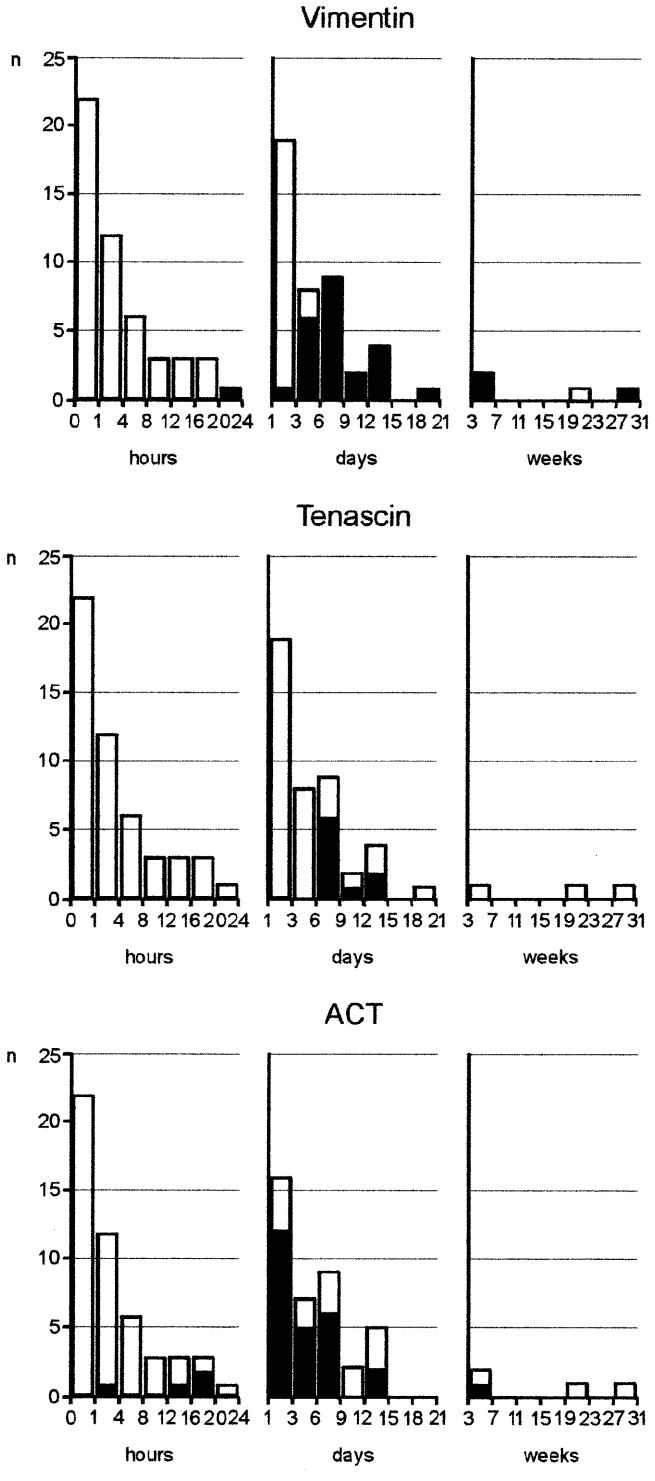

Fig. 3 Number of cases with negative (*white columns*) and positive (*black columns*) glial immunoreactivity for vimentin ($n = 104$), tenascin ($n = 103$) and α1-ACT ($n = 93$) related to the wound age

glial cells located adjacent to the damaged lesion exclusively.

The results indicating the course of the glial expression of the different immunohistochemical markers during the wound healing process after brain injury in humans are demonstrated in Fig. 3.

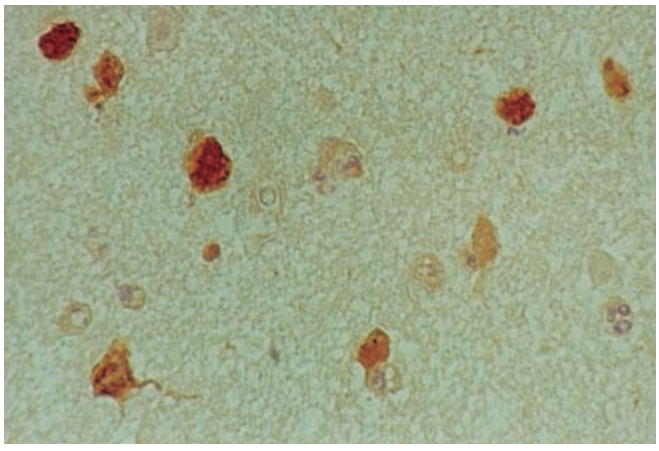

Fig. 4 Glial ACT expression adjacent to a cortical contusion with a wound age of 2 days (× 410)

Discussion

Reactive astrocytes are thought to play an essential role in the healing phase following CNS injury induced by actively monitoring and controlling the molecular and ionic contents of the extracellular space of the CNS [14]. Astrogliosis is also characterised by rapid synthesis of intermediate filaments, finally forming a glial scar [15, 23]. Previous studies concerning the glial fibrillary protein (GFAP) expression after human brain injury provided evidence of significantly increased GFAP immunoreactivity 1 day and up to 4 weeks after the trauma [18]. These findings have been thought to be of considerable interest for the timing of cortical contusions in forensic casework. As various other molecules have been shown to be modulated in glial cells after injury in experimental studies [29], it was of interest to determine whether time-related changes in the glial immunoreactivity could provide further information on the age of cortical contusions in human brain tissue. For this purpose, this study focussed on the immunohistochemical detection of vimentin, tenascin and α1-ACT during the wound healing process after the injury.

Vimentin is a major cytoskeletal component in the immature glia. It is considered to be expressed by radial glia and immature astrocytes early in development of the CNS and is replaced by fibrillary acidic protein (GFAP) during maturation [26]. According to the results of immunohistochemical studies, adult astrocytes appear to recover the capacity to express vimentin. Furthermore, the co-expression of GFAP and vimentin by reactive astrocytes has been described in experimental models during the first 2 post-lesional weeks [30]. Other authors found vimentin-positive astrocytes 5 days [10] or 7 days [34] after injury at the earliest. In accordance with these data, the present study demonstrated elevated astroglial vimentin expression in cortical lesions with a wound age of at least 6 days and up to 4 weeks after trauma. In addition, some cases of traumatic brain injury with a survival time between 22 h and 6 days showed vimentin-positive cells, characterised by large, round-shaped cell bodies and a lack of ramified

processes. Such cytological features are typical for brain macrophages, which have long been thought to derive from monocytic blood cells exclusively. However, it could be demonstrated that a fraction of the macrophages also corresponded to sessile microglia, which can be activated by all types of tissue damage involving neuronal injury. The activation is accompanied by morphological changes and up-regulation of different macrophage-associated antigens, which makes it difficult to distinguish activated microglia from monocytic cells [33]. Furthermore, there is evidence that activated microglia newly express the intermediate filament protein vimentin [16].

Tenascin is an extracellular matrix protein which is synthesised and released by immature astrocytes during embryonic and early postnatal development of the CNS [5, 6, 11, 12, 20, 24, 25, 32]. Whereas in the adult nervous system, tenascin can be detected only at very low levels, it has been shown that stab wounds to the adult mouse cerebellar and cerebral cortices resulted in an enhanced expression of tenascin in a discrete region around the lesion site that is associated with a subset of GFAP-positive astrocytes [9, 22]. In the human brain, localised up-regulation of tenascin was demonstrated by Brodkey et al [9]. The authors described a dense tenascin immunostaining in extracellular areas as well as some cellular staining (presumably astrocytes), which was located around a gun-shot wound with a survival time of 96 h.

In accordance with the findings reported in the literature, tenascin could not be detected in uninjured human brain tissue in this study. Increased tenascin expression was, however, evident following brain injury, and about 75% of cases with cortical lesions aged between 7 and 14 days showed a distinct staining of glial cells exclusively located around the damaged area. As described in the literature, the tenascin-positive glial cells were associated with the GFAP-positive astrocytes at the lesion site [22]. The findings suggest that tenascin up-regulation in the lesioned adult brain may be directly involved in failed regeneration or indirectly involved through interaction with other glycoconjugates that either inhibit or facilitate neurite growth, as discussed by Laywell et al. [22].

The serine protease inhibitor α1-ACT is an acute-phase protein which is present in the amyloid plaques that form the pathologic hallmark of Alzheimer's disease (AD) [1, 3, 31]. Studies using in situ hybridisation indicated that the α1-ACT found in AD plaques is produced primarily by astrocytes [21, 27]. Reactive astrocytes expressing α1-ACT have also been found in other neurologic diseases, including Huntington's chorea, Parkinson's disease and ischemic infarction of the brain [2]. Recently it was demonstrated that the expression of α1-ACT by reactive astrocytes can also be induced acutely in mice by focal injury [3]. This finding is confirmed by our immunohistochemical investigations, where α1-ACT positive glial cells were observed 3 h after the trauma at the earliest, and in the post-infliction interval ranging between 1 and 13 days the majority of cases (about 69%) were positive for α1-ACT. In about 10% of cortical contusions, the assessment of the immunostaining was complicated by high background staining. This phenomenon was also observed by other authors and was thought to be related to brain edema and/or other non-specific pathophysiological changes [3]. As described for the glial molecules, the α1-ACT expression was limited to the area around the cortical lesion, whereas no α1-ACT immunoreactivity could be observed at a distance from the contusions or in the uninjured brain tissue from the control group. These results support the assumption that increased α1-ACT expression may represent a consistent component of the astroglial response to neural injury [3]. However, the precise molecular mechanism by which astrocytes are induced to up-modulate α1-ACT expression remains unclear. In the liver, α1-ACT is strongly induced by interleukin-1 (IL-1) [7]. Hence, it seems possible that the injury-induced α1-ACT expression by reactive astrocytes may also be mediated by IL-1.

In conclusion, the present study clearly demonstrated a time-dependent expression of different glial molecules after mechanical injury in human brain, as described for other parameters which indicate the course of inflammatory reactions [17] or GFAP immunoreactivity [18]. In contrast to the GFAP staining reaction, the parameters investigated in this study showed no positive glial reaction in uninjured brain tissue, whereas positive results could regularly be observed adjacent to cortical contusions. Thus, the immunohistochemical detection of vimentin, tenascin and α1-ACT may be of value in the forensic age estimation of human cortical contusions.

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