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ORIGINAL ARTICLE

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A quantitative immunohistochemical study on the time-dependent course of acute inflammatory cellular response to human brain injury

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Abstract The time-dependent inflammatory cell reaction in human cortical contusions has been investigated during the first 30 weeks after blunt head injury. Immunohistochemical staining was carried out using CD 15 for granulocytes and LCA, CD 3 and UCHL-1 for mononuclear leucocytes. In order to provide reliable data for a forensic wound age estimation, the intensity of the cellular reaction was evaluated with a quantitative image analysis system. CD 15-labelled granulocytes were detectable earliest 10 min after brain injury, whereas significantly increased numbers of mononuclear leucocytes occurred in cortical contusions after a postinfliction interval of at least 1.1 days (LCA), 2 days (CD 3) or 3.7 days (UCHL-1), respectively.

Key words Brain injury · Cortical contusion · Inflammatory reaction · Immunohistochemistry · Wound age

Introduction

The acute inflammatory response is a stereotyped nonspecific reaction of tissues and myelomonocytic leucocytes to a variety of insults. The kinetics of cellular recruitment have been extensively investigated in different non-neuronal tissues and show a similar course. In the central nervous system (CNS) the cellular accumulation including neutrophils and mononuclear macrophages is thought to be implicated in the pathogenesis of secondary brain injury after trauma, specifically contributing to alterations in cerebral blood flow, edema, intracranial hypertension, and ultimately neuronal death [6, 18]. As information on the course of the post-traumatic cellular reaction may contribute to a forensic wound age estimation [7, 12–14], the time-dependent appearance of polymorphonuclear and mononuclear leucocytes positively staining for CD 15, LCA, UCHL-1 and CD 3 was investigated in human cortical contusions by immunohistochemistry.

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Material and methods

A total of 104 individuals aged between 6 and 81 years (average individual age 44 years) with closed head injury leading to macroscopically detectable cortical contusions was investigated. Secondary haemorrhages or disturbances of blood coagulation were not evident according to anamnestical data. Furthermore, there were no clinical or morphological indications of intravital interruption of intracerebral circulation for a longer period of time. The postinfliction interval ranged between a few minutes and 30 weeks and the postmortem interval did not exceed 3 days. All individuals with a survival period up to 3 weeks died from cerebral dysregulation caused by neuronal damage or its secondary complications, while in the remaining cases natural causes of death (e.g. acute coronary insufficiency) were found. At autopsy, injured brain as well as tissue samples of macroscopically unalterated cerebral regions were obtained in each case. Furthermore, brain tissue from 30 individuals without head injury who died from acute cardiac arrest (n = 10), SIDS (n = 5) traumatic asyphyxia (n = 10) or carbon monoxide intoxication (n = 5), acted as controls.

After fixation in 4% PBS-formaldehyde solution the tissue samples were embedded in paraffin and tissue sections (3–5 μm) were stained with hematoxylin eosin (H.E.). In addition, the following leucocyte antigens were detected by immunochemistry using the avidin-biotin-complex (ABC) method: LCA (= CD 45, DAKO, # F 0861), UCHL-1 (= CD 45 RO, DAKO, # T 1067), CD 3 (DAKO, # A 0452) and CD 15 (DAKO, # V 1615). Enzyme pretreatment with pronase 0.1% (CD 3 and UCHL-1) or with trypsin (LCA) was applied. The primary antibodies were used at a dilution of 1 : 50 (CD 15), 1 : 150 (CD 3), 1 : 400 (LCA) or 1 : 1000 (UCHL-1).

Only those leucocytic cells showing a distinct cellular staining reaction were regarded as positive. Specimens which showed autolytic changes such as post-mortem cell shrinkage or diminished nuclear stainability in H.E. stained preparations were excluded.

The evaluation also considered different topographic localisations of the leucocytes such as:

1. Predominantly perivascular mononuclear cells / granulocytes
2. Recruitment of inflammatory cells adjacent to the cortical contusion and outside of the haemorrhagic area
3. Mononuclear cells and granulocytes in the subarachnoidal space.

A quantitative analysis was performed using an automatic image processing and analysis system (LEICA QWin). For data evaluation, the total number of positively stained cells in each topographic region was determined within a microscopic measuring frame of 0.125 mm^2 (objective 16/0.40, occular × 10). The average cell number in three microscopic fields was defined as an average score. Mean values of at least 5.0 labelled mononuclear cells per defined area were considered as a positive reaction with respect to the comparable low numbers of positively stained single cells which were also found in the uninjured brain tissue of the control cases. Since the CD 15 reaction was negative in all control cases (n = 30), the determination of at least 1 stainable granulocyte/microscopic field adjacent to the cortical contusion could theoretically be considered as a positive reaction.

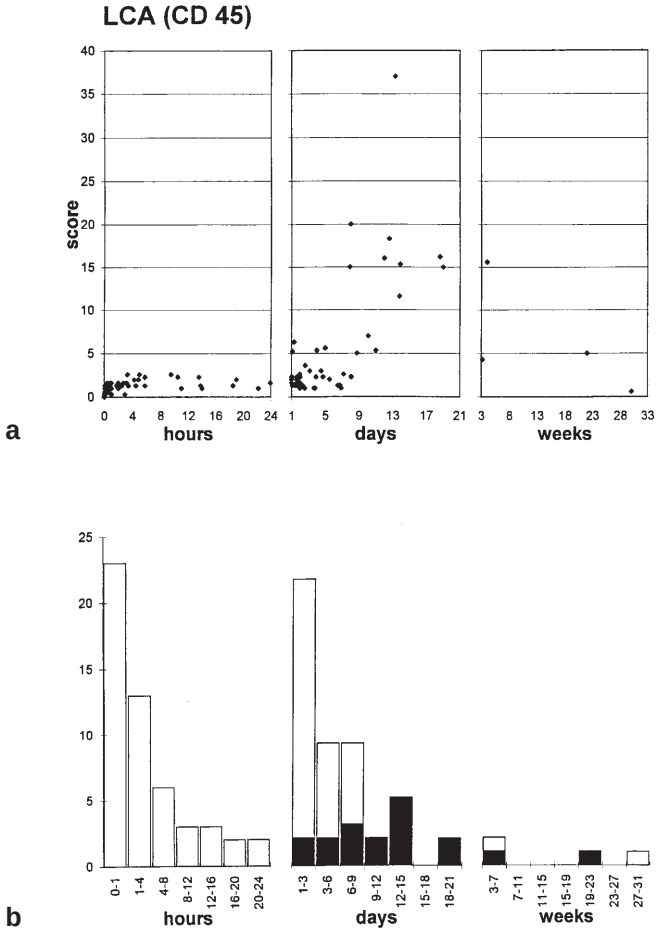

Fig. 1 a, b Mean values (score) of LCA labelled mononuclear leucocytes in traumatically injured brain tissue related to the wound age (**a**). Number of cases with at least five (black columns) and less than five (white columns) positively stained leucocytes in each age group (**b**)

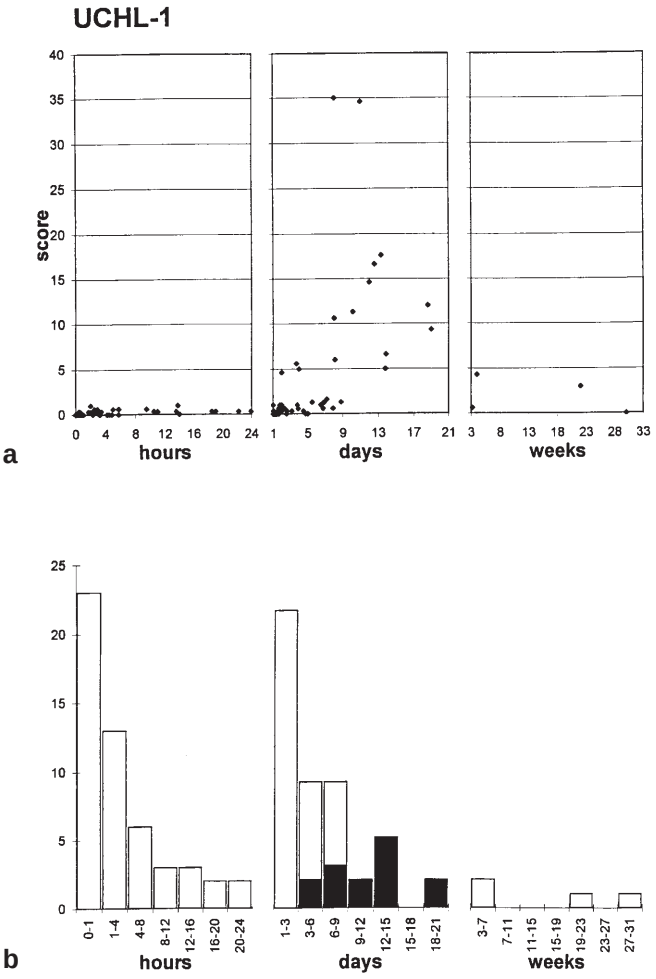

Fig. 2 a, b Mean values (score) of UCHL-1 labelled mononuclear leucocytes in traumatically injured brain tissue related to the wound age (**a**). Number of cases with at least five (black columns) and less than five (white columns) positively stained leucocytes in each age group (**b**)

Results

LCA

According to the manufactures specification sheet the DAKO anti-leucocyte common antigen labels the cell membrane of lymphoid cells. Macrophages and histiocytes react to a variable degree with the antibody. Polymorphs are usually only weakly labelled, while many plasma cells are unreactive. The antigen on the surface of leucocytes defines a family of high molecular weight glycoproteins.

Uninjured brain tissue

Single LCA-positive stained leucocytes were detectable in uninjured neuronal parenchyma in 26 out of 30 control cases. The average numbers of positively stained cells (scores) reached values up to 0.6 in cases of acute cardiac

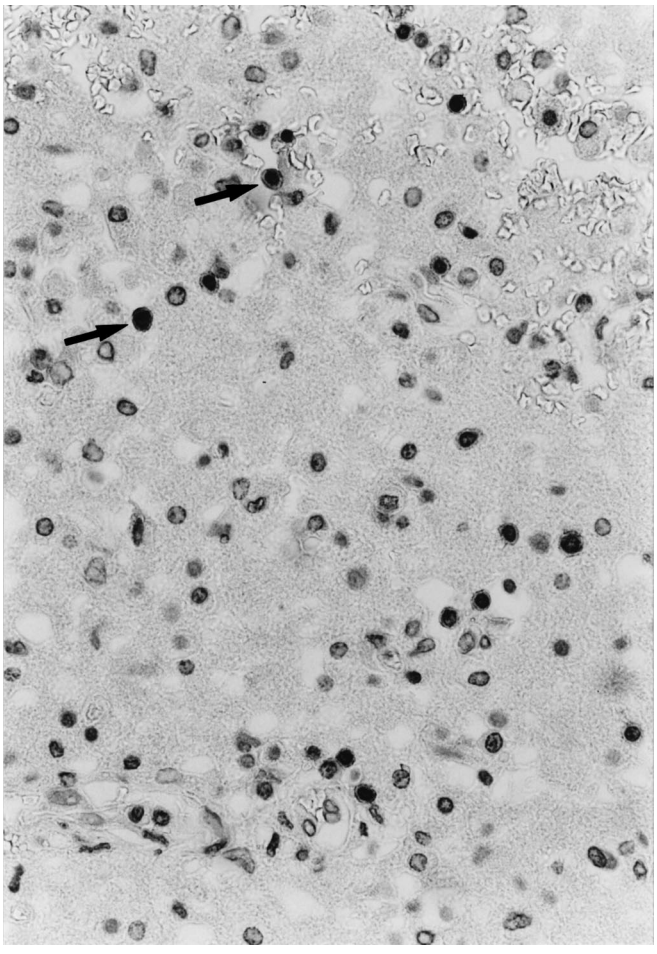

Fig. 3 Mononuclear leucocytes showing a distinct positive reaction with the CD 3 antibody (arrows) in a cortical lesion 10 days post injury (× 300)

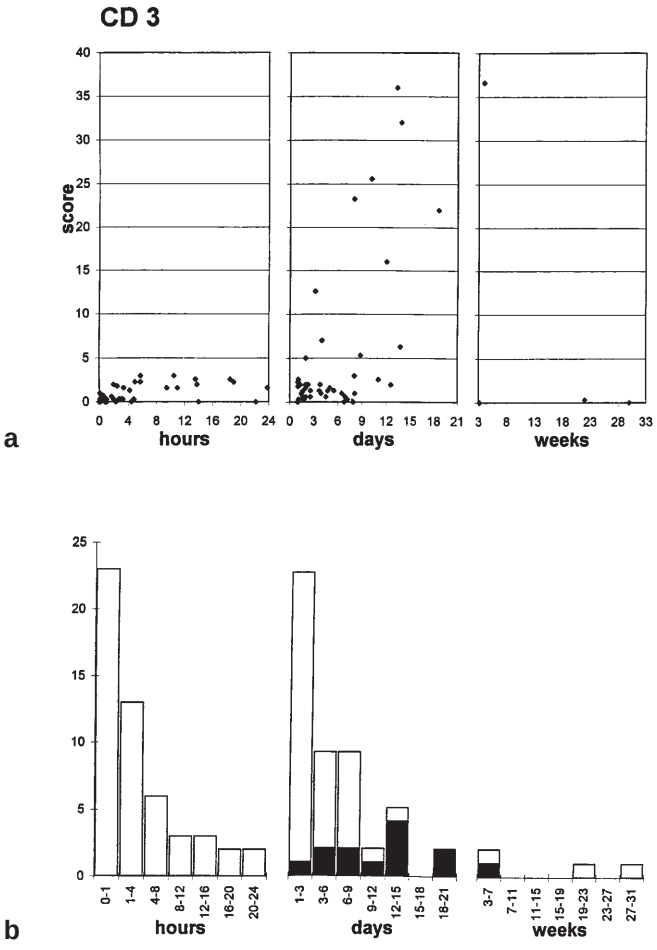

Fig. 4 a, b Mean values (score) of CD 3 labelled mononuclear leucocytes in traumatically injured brain tissue related to the wound age (**a**). Number of cases with at least five (black columns) and less than five (white columns) positively stained leucocytes in each age group (**b**)

death, 0.3 in infants dying of SIDS, 1.3 in traumatic asphyxia and 1.0 in cases of carbon monoxide intoxication. As positively stained cells were regularly found in the perivascular and subarachnoidal spaces in comparably high numbers, the examination of the inflammatory reaction in damaged brain tissue was confined exclusively to the cortical contusion.

Cortical contusions (Fig. 1)

In lesions with a postinfliction interval of a few minutes up to 24 h, the average values of positively stained lymphoid cells adjacent to the cortical contusion ranged between 0.0 and 2.6. Polymorphonuclear granulocytes, which could also be identified in some of these cases, showed a comparatively weak staining reaction and were therefore not regarded as positive cells. Single labelled cells (score < 0.3) were even seen in cortical wounds with short survival intervals of a few minutes as well as in macroscopically unaltered sections from the same individual. The threshold value of 5.0 cells / microscopic field, defined as a positive reaction, was exceeded earliest in a wound aged 1.1 days. In the postinfliction interval between 9 and 21 days, LCA positive cells were regularly found with mean values ranging between 5.3 and 37.0 cells per defined area. Positive LCA reactions were also detected in cortical lesions with advanced postinfliction intervals (wound age up to 4 weeks, score 15.6).

UCHL-1

This antibody specifically recognizes the 180 kD low molecular weight isoform of CD 45 and stains a subpopulation of resting T-cells within both the CD 4 and CD 8 subsets. It occurs on most thymocytes and activated T-cells. Most normal B-cells and NK-cells are negative.

Uninjured brain tissue

In 5 out of 30 control cases, very few positively stained leucocytic cells were detectable in cerebral parenchyma. The maximum mean value was 0.6 in a case of acute car-

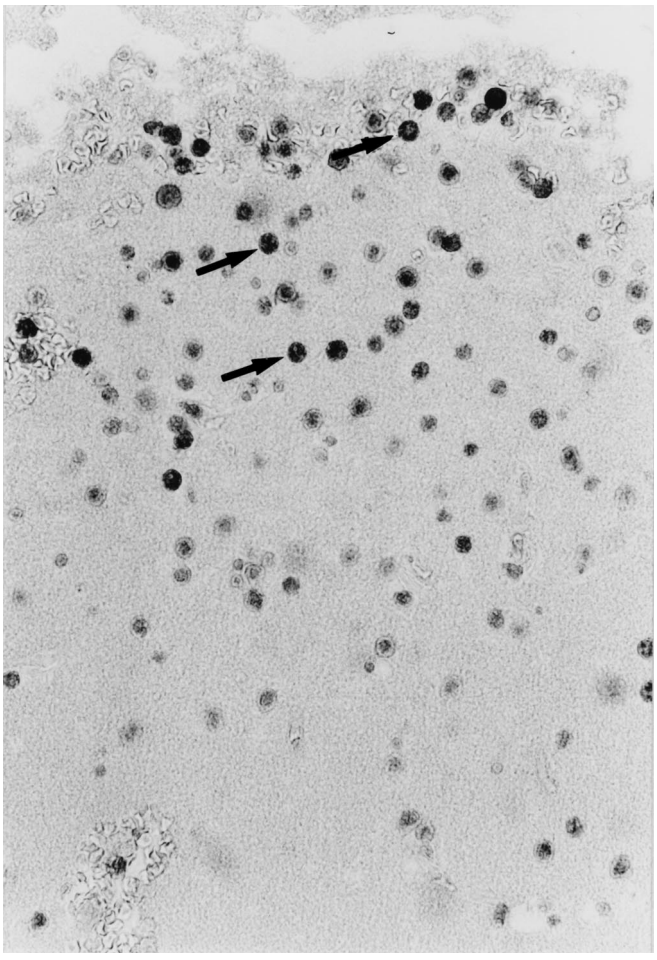

Fig. 5 CD 15 positively stained polymorphonuclear leucocytes (arrows) adjacent to the damaged neuronal parenchyma in a cortical contusion with a postinfliction interval of 1.5 days (× 300)

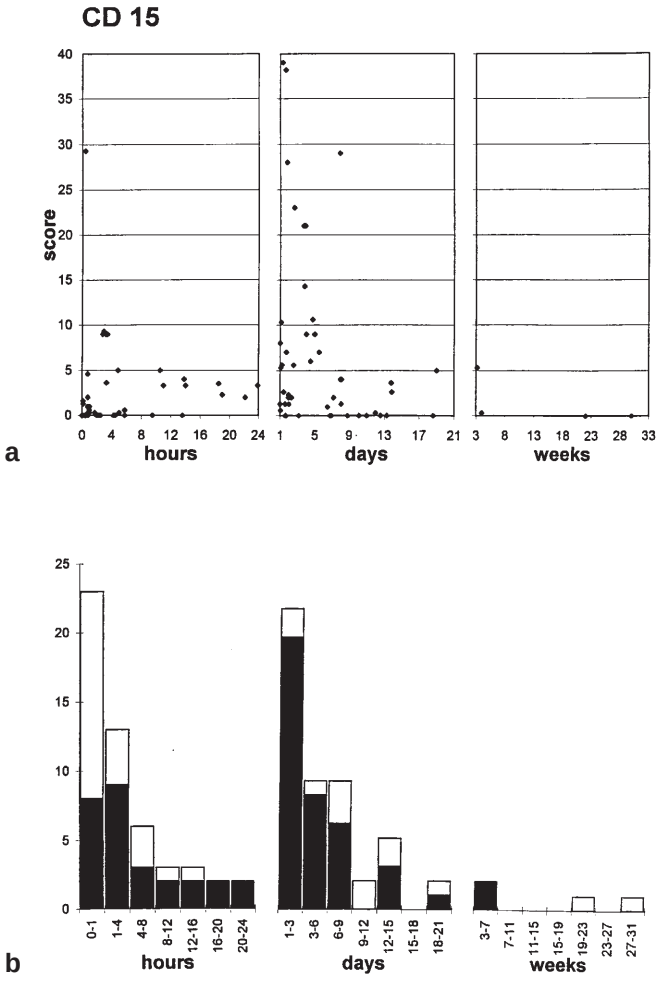

Fig. 6 a, b Mean values (score) of CD 15 labelled polymorphonuclear leucocytes in traumatically injured brain tissue related to the wound age (**a**). Numbers of cases with at least 1 positively stained leucocyte/microscopic field (black columns) in each age group (**b**)

diac arrest. The median values of UCHL-1 positive cells in the perivascular and subarachnoidal spaces reached 4 cells / microscopic field for each topographic region.

Cortical contusions (Fig. 2)

In cortical traumatic lesions with a postinfliction interval up to approximately 20 min, no UCHL-1 positive cells were found adjacent to the region of neuronal damage (n = 14). Comparably low numbers of positively stained leucocytic cells (score < 1.0) were seen in cortical contusions not older than 1 day. A positive reaction was obtained in lesions with a wound age of at least 3.7 days and was almost regularly be found in specimens with postinfliction intervals ranging between 10 and 19 days. In wounds older than 3 weeks, no significant number of UCHL-1 positive leucocytes occurred.

CD 3

The CD 3 antigen is a lineage-specific 'pan T-cell' surface antigen and is normally present on mature thymocytes, resting and activated peripheral T lymphocytes (both inducer and supressor/cytotoxic population) and some natural killer cells. The antigen is not detectable on peripheral blood B lymphocytes, monocytes, granulocytes or platelets. Surface expression of CD 3 is preceeded by its occurrence in the cytoplasm of immature and common thymocytes.

Uninjured brain tissue

CD 3 was found to be expressed only by single leucocytic cells located in the cerebral parenchyma in 7 out of 30 control cases (score < 0.3), whereas in the paravascular space up to 4 cells/microscopic field could be detected.

Cortical contusions (Figs. 3, 4)

The earliest positive reaction was observed in a traumatically injured brain with a postinfliction interval of 2 days (score = 5.0). In 8 out of 10 cortical lesions aged between

9 and 19 days, the mean values ranged between 5.3 and 36.0. Cortical contusions older than 22 weeks showed no significant number of CD 3 positive mononuclear cells.

CD 15

CD 15 is present in neutrophil secondary granules of granulocytic cells which express the antigen late in their maturation.

Uninjured brain tissue

Polymorphonuclear leucocytes were detectable neither in neuronal parenchyma nor in the perivascular or subarachnoidal spaces of uninjured brain tissue.

Cortical contusions (Figs. 5, 6)

CD 15 positive polymorphonuclear leucocytes (PMN) were detectable as early as 10 min after brain damage (score 1.6). In a cortical contusion which was survived for 25 min, a comparably high number of positive PMNs (score 29.3) was observed. In cerebral wounds aged between 14 h and 1.6 days, positively stained leucocytes occurred regularly in all cases, reaching mean values of 38.2 cells/field, and some neutrophil infiltrates were visible up to 4 weeks after brain injury (score 0.3). In the subarachnoidal and perivascular spaces, no relevant granulocytic recruitment could be detected.

The results of the morphometric analysis are demonstrated in Table 1.

Discussion

Cerebral contusions are characterized by perivascular haemorrhages orientated perpendicularly to the cortical surface and edema in the surrounding brain which is followed by tissue necrosis. Some clinical studies found circumstantial evidence of an influence of polymorpho- and mononuclear cell infiltrates for deterioration of the initial tissue damage [10]. Inflammatory cells are also involved in the growth and repair of damaged parenchyma after brain injury [8] and the time-dependent appearance of different leucocyte subtypes can furthermore contribute to a forensic wound age estimation.

The course of reactions to cerebral traumatic contusions has been studied over the years regarding early processes, such as traumatically induced edema or neuronal damage and to late reactions, specifically in relation to the developement of the macrophage (microglial) response [14, 15], the evolution of the glial scar and the fate of blood pigments. On the other hand, comparatively few reports exist on the course of the inflammatory cellular response to cortical contusions in human brain tissue and in animals. In contrast to peripheral tissue the cellular reaction in the CNS was found to be characterized by a minimal neutrophil exsudation and a delayed increase in mononuclear cell numbers [1, 10, 16]. Protective mechanisms of the CNS parenchyma, such as the presence of the blood-brain-barrier and the specialized nature of the cerebral endothelium or the down-regulated microglial activity might be responsible for this comparably late inflammatory response in the brain [1].

Concerning the velocity of the leucodiapedesis, varying results have been reported in the literature. According to the findings of experimental studies in rats, early neutrophil accumulation within the first 24 h after brain injury, as measured by myeloperoxidase activity (MPO), was thought to indicate the beginning of inflammatory reaction [4, 6, 11, 18]. Histological investigations of experimental brain injuries, however, revealed no relevant granulocytic recruitment [1, 10, 15], whereas in other morphological studies some polymorphonuclear granulocytes were detectable in damaged cortex 4 h after the trauma [19]. Polymorphonuclear infiltrations adjacent to the necrotic neuronal parenchyma of rats were present earliest at day 2 and decreased further with time [10]. In human brain tissue polymorphonuclear leucocytes have been found within a few hours of injury [5, 12, 14, 17], emanating from vessels and invading the damaged tissue [5]. In contrast to these findings we found a rather early leucocyte reaction adjacent to the damaged cortical area by immunohistochemical staining. Considerable numbers of CD 15-labelled polymorphonuclear leucocytes were found earliest in a cortical lesion with a postinfliction interval of 10 min. Maximum cell numbers occurred within the first 2 days after brain injury and according to other studies [5, 15] infiltrations were visible up to 4 weeks even though no infection was present.

With regard to the mononuclear cellular response, a diffuse lymphoid reaction has been reported 3 or 4 days at the earliest [5, 12] and up to 44 years after brain injury [12]. In experimental brain contusions in animals, an in-

Table 1 Average numbers (mean values) of positively labelled polymorpho- and mononuclear leucocytes in cortical contusions compared to the positive reaction in uninjured brain tissue

Antigen	Specificity	First positive reaction	Regular positive reaction	Mean values (scores) of positively stained cells in	
				cortical contusions	uninjured brain tissue
CD15	neutrophile granulocytes	10 min	14 h–1.6 d	1–39.0	0.0
LCA	leucocytes	1.1 d	9–21 d	5–37.0	0.0–1.3
CD 3	T-cells (inducer, supressor, NK)	2 d	13.3–19 d	5–36.6	0.0–0.3
UCHL-1	T-cells (CD4/CD8)	3.7 d	10–19 d	5–35.0	0.0–0.6

flammatory mononuclear cell response was evident on day 2 with a maximum on days 5 and 6 and signs still remained 16 days after the trauma. Most inflammatory cells have proved to be activated T-cells that may have receptor-mediated cytotoxic or modulating effects on cells in the CNS [10, 20].

The primary antibodies UCHL-1 and CD 3 used in this study, also detect some subpopulations of T-cells including CD 4/CD 8 subsets, inducer and supressor/cytotoxic cells and some natural killer cells. Significant numbers of T-cells labelled by CD 3 were detectable locally in cerebral lesions after a posttraumatic interval of at least 2 days. UCHL-1 positive lymphocytes occurred earliest 3.7 days after the trauma and the leucocyte common antigen showed a positive reaction in traumatized brain tissue with a wound age of at least 1.1 days. In accordance with the manufacturer's information, the LCA mainly labelled mononuclear lymphoid cells in our study, whereas the majority of polymorphonuclear leucocytes showed only a weak staining reaction which was not considered for evaluation as positive. Therefore, distinctly lower score values of positively stained leucocytes could be determined in wounds with short survival intervals when compared to the CD 15 reaction.

Since some mononuclear cells staining with the LCA-, CD 3- and UCHL-1 antibodies were found near blood vessels of the neuronal parenchyma as well as in the subarachnoidal space of uninjured brain in the control cases, only the evidence of cellular infiltrates adjacent to the damaged neuronal parenchyma can provide reliable information for a wound age estimation. It must be discussed whether such perivascular accumulations of mononuclear leucocytes represent a unspecific reaction due to different natural or traumatic causes of agonal oxygen deficiency in the brain tissue. Furthermore, it should be noted that intravascular accumulations of leucocytes can also occur in passive haemodynamic congestion and do not prove an active cellular response to tissue damage.

Although the results showed an intra- and interindividual variability as expected principally in studies dealing with wound age estimation [3, 9], the immunohistochemical detection of inflammatory cells may be a valuable parameter for the age estimation of human cortical contusions. To avoid a misinterpretation of immunohistochemically findings a critical evaluation is necessary [2]. Taking these aspects into consideration, the following conclusions can be drawn:

1. Polymorphonuclear cells were detectable earliest in cortical contusions after a postinfliction interval of 10 min.
2. A positive reaction for the leucocyte common antigen (LCA) indicate a wound age of at least 1 day.
3. Significantly increased numbers of mononuclear leucocytes were found in cortical lesions with a wound age of at least 2 days (CD 3) or 3.7 days (UCHL-1).

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