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The neuroinflammatory response in humans after traumatic brain injury

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Abstract

Aims—Traumatic brain injury is a significant cause of morbidity and mortality worldwide. An epidemiological association between head injury and long-term cognitive decline has been described for many years and recent clinical studies have highlighted functional impairment within 12 months of a mild head injury. In addition chronic traumatic encephalopathy is a recently described condition in cases of repetitive head injury. There are shared mechanisms between traumatic brain injury and Alzheimer's disease, and it has been hypothesised that neuroinflammation, in the form of microglial activation, may be a mechanism underlying chronic neurodegenerative processes after traumatic brain injury.

Methods—This study assessed the microglial reaction after head injury in a range of ages and survival periods, from <24 hours survival through to 47 years survival. Immunohistochemistry for reactive microglia (CD68 and CR3/43) was performed on human autopsy brain tissue and assessed "blind" by quantitative image analysis. Head injury cases were compared to age matched controls, and within the traumatic brain injury group cases with diffuse traumatic axonal injury were compared to cases without diffuse traumatic axonal injury.

Results—A major finding was a neuroinflammatory response which develops within the first week and persists for several months after TBI, but has returned to control levels after several years. In cases with diffuse traumatic axonal injury the microglial reaction is particularly pronounced in the white matter.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Conclusions—These results demonstrate that prolonged microglial activation is a feature of traumatic brain injury, but that the neuroinflammatory response returns to control levels after several years.

Keywords

neurotrauma; microglia; neuroinflammation; traumatic axonal injury

Introduction

There is a considerable retrospective epidemiological literature suggesting that traumatic brain injury (TBI) is associated with an increased risk of developing Alzheimer's disease (AD) in later life [1, 2] although not all studies have confirmed this association [3]. Data from prospective studies has also been conflicting with some studies showing an association [4] while others show no association [5]. Meta-analysis of 7 case-control studies [6] calculated a relative risk of developing AD of 1.82 for head injury with loss of consciousness, only reaching statistical significance for males. Fleming *et al.* [7] studied 15 case-control studies and calculated an odds-ratio (OR) of 1.58. Again, however, this study showed that the association between head injury and AD was only statistically significant for males (males OR 2.26, females OR 0.92) the group that form the majority of the head-injured population.

Follow up of patients 10–20 years after admission to hospital with TBI provided further evidence of late stage neurodegeneration [8]. Even mild head injury (acute Glasgow Coma Score [GCS] 13–15) is associated with a higher than expected incidence of disability (Glasgow Outcome Score: moderate or severe disability) at one year post injury [9].

The data relating to repetitive head injury is more secure. Dementia pugilistica has been known for many years [10], although is a rare condition, with cerebellar and parkinsonian phenotypes being seen more commonly than overt dementia. Tau deposition is described although in a distribution that differs from AD [11], and the tau isoform is the same as seen in AD [12]. Recent studies have focused on the idea of a specific neurodegenerative condition after repetitive head injury, chronic traumatic encephalopathy (CTE) [13]. The concept of CTE has been extended beyond cognitive problems to motor disorders, with pathological TDP-43 deposition being described in 3 CTE cases and linked to an amyotrophic lateral sclerosis (ALS)-type disorder [14], although many researchers have objected to this term [15,16]. Studies looking at outcomes after a single head injury using this Glasgow cohort could not demonstrate tau deposition in acute head injury with a survival time of up to 1 month [17], but a higher incidence of tau and β -amyloid (A β) deposition has recently been described with survivals ranging from 1–47 years, compared with age-matched controls [18], providing further evidence of an association between TBI and AD.

Previous studies have focused attention on neuroinflammation in the form of microglial activation, as a mechanism of potential relevance to neurodegeneration both in (AD) and in the response to brain injury [19,20,21,22]. Microglial phenotypes may be modified by an external or intrinsic stimulus, and undergo morphological changes and release proteins which may be detrimental or beneficial to the surrounding brain tissue [23]. When activated the microglia can change their morphology and can express new cell surface markers, or alter expression of pre-existing markers, including the MHC class II antigen [24] and a marker of phagocytic activity, CD68 [25].

After brain injury cytokines are released activating microglia, the degree of activation reflecting the severity of the injury [26]. Microglial activation will lead to further cytokine

release, including IL-1, possibly secondary to elevated levels of ATP released from damaged cells [27], with activation of purinergic P2X7 receptors on microglia [28]. IL-1 is expressed in increased quantities in the cerebral cortex within hours of TBI [29], and chronic overexpression of IL-1 is found in AD [30]. Griffin *et al.* [21] have proposed a “Cytokine Cycle” in which traumatic brain injury, or other forms of brain injury, can, in susceptible individuals, initiate an over-exuberant sustained inflammatory response which can result in neurodegeneration. IL-1 positive microglial cells lie in close relation to β -APP positive neurons and dystrophic neurites in the brains of head-injured patients [29] and are also found in close apposition to neurofibrillary tangle-containing neurons in AD [30]. β -APP is up-regulated in response to increased IL-1 levels, and is known to be up-regulated in AD [31,32].

In an attempt to test the hypothesis that the microglial response to head injury may persist and provoke chronic neurodegeneration, we have characterised in detail the time course and magnitude of the microglial reaction in human post mortem cases.

Materials and methods

Case selection

The study was approved by the Research Ethics Committee of the Southern General Hospital, Glasgow, Scotland. Cases were selected from the paraffin-embedded tissue archive of the Neuropathology department, Institute of Neurological Sciences, Glasgow. This is a unique archive of more than 1500 well-characterised TBI cases, with a range of survival periods. The acute (less than 12 months survival) cases used in the current study were selected from a cohort accrued between 1987–1999, and the long-term survivors were selected from the entire cohort (1968–1999). 57 cases were selected from the 1987–1999 cohort to represent the <12 months survival group, cases being selected based on completeness of clinical information and available tissue blocks. Ages ranged from 1.5–89 years, and there were 39 males and 18 females. 42 long-term survivor cases were identified from the archive again based on available clinical information and tissue availability. Ages ranged from 19–89 years and there were 37 males and 5 females. This resulted in a total of 99 TBI cases with varying survival times ranging from <24 hours up to 47 years being included in this study (Tables 1 and 2). Of the 57 cases that survived < 1 year, 45 cases had experienced a severe head injury, 34 cases defined by their admission GCS (GCS 8 or less) and 11 defined on review of the clinical history (patients died rapidly before hospital admission and pathology suggested a head injury was a major feature of the autopsy findings). In 3 cases there had been a moderate injury based on admission GCS (GCS 9–12) and 3 cases had had a mild head injury (GCS 13–15) and died of pathology not directly related to the brain injury. In 6 of the longer survivors in the 12 month survival group there was no data relating to the GCS at time of admission although review of the clinical data again suggested a severe head injury with coma since the time of injury (survival ranging from 5 weeks to 1 year in these cases). For the 42 cases with survival beyond 1 year, admission GCS data was not available for the majority of cases. The mechanisms of injury varied with road traffic accidents (RTA) being more common in the <20 year old group, and falls more common in the >50 year old group. Post mortem interval data was available for all cases and ranged from 24–60 hours.

20 cases without TBI and with no other significant neurological impairment or neuropathological abnormality were used as controls (Table 1). Ages ranged from 18–71 years and there were 12 males and 8 females. These patients all had hospital post mortem examinations which were fully consented. Tissue was retained for diagnostic purposes with full neuropathological examination including neurohistology. The cause of death in the control cases is detailed in Table 3.

All cases were coded such that assessment was “blind”.

Macroscopic brain examination and sampling for histology

The brains had been fixed in 10% formal saline for 3 weeks prior to dissection after which a brain cut and histological sampling was undertaken. No brains were subjected to prolonged formalin fixation. The brains were cut at a 1-cm thickness in the coronal plane. Full macroscopic and microscopic examination was undertaken in each case. The histological sections examined from each case in this study were the parasagittal cortex including corpus callosum, internal capsule and hippocampus, all sampled at the level of the lateral geniculate body; the cerebellum including dentate nucleus; and the pons including cerebellar peduncles. This represents the minimum recommended sampling when assessing diffuse traumatic axonal injury (TAI) [43]. The tissue was processed in a VIP tissue processor (Bayer Diagnostics, Newbury, UK) using a 60-hour cycle and embedded in paraffin wax. 8 µm thick sections of the paraffin blocks were cut and stained with haematoxylin and eosin (H&E). For the assessment of TAI a monoclonal antibody against the N-terminus of the human APP molecule (clone 22C11, Böehringer, dilution 1:50) was used, and TAI was documented as being absent or present.

Immunohistochemistry for activated microglia

Immunohistochemistry was undertaken for markers of microglial activation. Sections were immunostained with anti-CD68 which binds lysosomes and therefore identifies microglia with phagocytic functions (mouse monoclonal antibody to a macrophage-specific 110 kDa glycoprotein -Dako, 1:1000) and CR3/43 which labels activated microglia (mouse monoclonal antibody to class II MHC: HLA-DR, -DQ and -DP chains - Dako, 1:800). The antibodies were detected using the ABC kit (Vecta Stain, Vector Laboratories, Peterborough, UK) and developed with DAB. In this study counterstaining with haematoxylin was weak to allow greater sensitivity of image analysis as discussed below.

Image analysis

Image analysis was undertaken to assess the immunostaining load within defined anatomical regions in cases of TBI and to compare these with control cases. The regions of interest were the hippocampus, the inferior temporal gyrus, and the corpus callosum and cingulate gyrus at the level of the lateral geniculate body. These areas were selected as they frequently show pathological changes in TBI cases.

The morphometric study used an image analysis system consisting of a digital CCD-Camera (CoolSnap-Pro®) linking an Olympus BX 40 Light Microscope to a PC with the image analysis software (Image-Pro® Plus, Media Cybernetics). Multiple non-overlapping colour images of the area of interest were captured using x10 stage objective lens and the images were tiled together automatically to give a large composite image. Lamp intensity, digital camera set-up and calibration parameters were kept constant throughout the capture of images.

The image analysis software (Image-Pro® Plus, Media Cybernetics) used in this study allowed the definition of immunoreactive profiles based on a defined threshold (segmentation). Segmentation is a process which allows the isolation of certain colours from an image as a whole. In this study the immunoreaction was developed by diaminobenzidine (DAB) which produces a brown precipitate. A manual segmentation technique was used to isolate the brown immunoreaction in the captured images. The sections were all weakly counterstained with haematoxylin to allow greater differentiation and to permit neuroanatomical orientation. The stored images were magnified to allow greater sensitivity during the segmentation process. The number generated by image analysis (immunoload) is

a reflection of the number of pixels with a colour determined by the threshold within the image analysed against the number of pixels not showing that colour pattern.

While this programme did allow the threshold setting to be applied as a constant to all images this was not done due to immunostaining intensity variation between batches of immunostaining. Therefore each slide had unique parameters set for segmentation based on the intensity of immunostaining. While this was more labour intensive it allowed greater sensitivity in the segmentation process. All results were generated by one analyst blind to clinical data to remove any inter-observer variation. To assess intra-observer variation, the same field of the same slide was analysed at the start of each session, and the load scores checked to see if they were similar. This showed less than 5% variation over a ten day period.

Data analysis

The project was designed as an observational study. For the purpose of analysis an individual's overall immunoload was obtained for each case by averaging the available values for each anatomical region. Immunostaining load is presented as a percentage of the total area multiplied by 10. Box and whisker plots showing the minimum, lower quartile, median, upper quartile, maximum and outliers(*) were used to display the data graphically. Actual data points were superimposed (°) on the boxplots. Overall immunoloads for groups were compared using a Kruskal Wallis test with Mann Whitney pairwise comparisons if differences were identified. In the pairwise comparisons a Bonferroni correction was applied to take account of multiple comparisons. Summary statistics for the individual areas are presented. All tests were two sided. Statistical significance was taken as $p < 0.05$. Minitab 16 was used for data analysis.

Results

CD68 and CR3/43 immunohistochemistry was undertaken on all 99 pre-selected cases for the neuroinflammation study. Microglial cells were seen with differing morphological appearances; these ranged from resting ramified cells to rounded phagocytosing amoeboid cells (Figure 1). There was considerable variation in CD68 and CR3/43 immunoreactivity within cases (Figure 2). Immunostaining was seen throughout the areas of interest. The pattern of staining was diffuse, with no specific anatomical pattern. There was no accentuation of immunostaining in association with regions described as being most susceptible to neurodegenerative pathology in CTE (subpial region, periventricular region, sulcal depths, or perivascular distribution) [13]. While post mortem interval and period of formalin fixation can influence immunohistochemical staining, no significant differences were seen in the groups studied with relation to these variables. All cases were handled in a standardised protocol with good post mortem refrigeration and relatively consistent post mortem interval of around 40 hours (range 24–60 hours). The cases with longer post mortem intervals (towards 60 hours) showed no less immunostaining compared to those cases with a shorter post mortem interval. No cases had a period of prolonged formalin fixation.

Effects of age on immunoload in control cases (Figure 3)

To examine the effect of age on overall immunoload all control cases under the age of 50 ($n=11$) and control cases over the age of 50 ($n=9$) were compared. There was no significant difference observed between the two groups for CR3/43 (median 1.41 vs 2.01, $p=0.27$). The median immunoload for each separate region is given in supplementary material and shows the values were higher in all regions including the temporal lobe and hippocampus, sites commonly involved in Alzheimer's disease. For CD68 the immunoload was less

pronounced although there was a significant reduction in the overall immunoload with ageing (median 0.36 vs 0.19, $p=0.04$).

Immunoload in Controls and TBI cases in relation to survival time (Figures 4 & 5)

Firstly, inflammation markers were compared in the controls, those who survived less than one year and those who survived more than 1 year (Figure 4). Both CR3/43 ($p = 0.02$) and CD68 ($p = 0.01$) showed statistically significant differences amongst the 3 groups. With CR3/43, those who survived >1 year (median 0.8) had lower levels than control cases (median 1.8) ($p = 0.05$) and those who survived <1 year (median 2.9) ($p = 0.04$). However, there was no statistically significant difference between controls and those surviving <1 year. With CD68, those who survived >1 year had lower levels (median 0.43) than those who survived <1 year (median 0.85) ($p = 0.02$) but neither was significantly different from control (median 0.42). Secondly a more detailed relationship with survival is shown in Figure 5. In these boxplots the median is highlighted by large solid dot. Broadly speaking the levels go down from control in the first 24 hours then rise with a maximum around 3 months, and then fall again. This is particularly noticeable in the corpus callosum (which has the highest levels of response of all 4 areas) but is also seen in the cingulate white matter (supplementary material).

Effect of the presence of traumatic axonal injury (TAI) on immunoload (Figure 6)

Within the group of 57 TBI cases with survival <1 year, 30 had a pathological diagnosis of TAI. Image analysis of CR3/43 of TAI cases when compared to non-TAI cases showed a higher overall immunoload (median 3.5 vs 1.8, $p = 0.03$). Immunoload was higher in all regions (supplementary material), but particularly in regions related to the white matter. CD68 also showed a significant increase in cases of TAI (median 0.90 vs 0.55, $p = 0.02$) although this was less pronounced than that seen for CR3/43. Again the median immunoload was higher in all regions (supplementary material). The findings were not related to focal pathologies such as contusions, haematomas and infarcts.

Discussion

This study has shown that there is a significant up-regulation of the neuroinflammatory response as identified with CD68 immunoreactivity after TBI when compared to non-trauma controls, and that this response peaks around 3 months survival but returns to control levels after several years. Within the group of TBI cases a difference was seen between cases of TAI and cases of non-TAI with both CD68 and CR3/43 being up-regulated in the cases of TAI. As with all autopsy based studies the majority of cases were from the severe TBI group with death being an end-point. It must be recognised that this group of patients may not necessarily be representative of TBI patient groups, and it is difficult to draw a direct correlation with long-term survivors of mild head injury.

Neuroinflammation in controls

Assessing the resting levels of MHC class II expression in human brains is difficult as even control (non-trauma) brains will have been subjected to some form of agonal event which may have resulted in microglial activation. A further confounding factor is the increasing realisation that inflammation within the CNS can be modified by systemic inflammatory responses [33,34]. Clinical studies of AD patients have demonstrated that impairment of cognitive function persists for up to 2 months after systemic infection and is associated with elevated serum levels of IL-1 [35]. Delirium is a well recognised consequence of systemic infection, and delirium is associated with increased microglial activation [36]. TNF in particular may have a role in activating microglia during systemic sepsis [37]. The cases used as controls in this study were defined as having no neurological disease during life and

no significant neuropathology demonstrated at autopsy. However, on review some of these cases died with pneumonia and others had systemic haematological malignancies or inflammatory conditions. It is therefore possible that some of the control cases had a neuroinflammatory response to systemic disorders such as infection and malignancy. Systemic disorders did not appear to affect the CNS in a predictable fashion such that some cases of systemic haematological malignancy were associated with increased microglial reaction while others were not. Future studies assessing the neuroinflammatory response may need to apply more stringent definitions to their control tissue.

Despite these limitations the age-matched control cases used in this study have results which suggest that MHC class II expression is normally low in the human brain and that while there is an age-related increase in MHC class II expression in temporal and hippocampal regions this did not reach statistical significance. These are regions frequently affected in AD and MHC class II expressing microglia have been described in relation to neuritic plaques [38,39] in AD, a condition predominantly associated with ageing. Phagocytic activity, as demonstrated by CD 68 showed a significant reduction with ageing.

Microglial activation in TBI

The most pronounced increase in MHC class II expression after TBI was seen in the central white matter regions (corpus callosum and cingulate gyrus) of cases which were diagnosed pathologically as having TAI. As expected, given that there would be Wallerian degeneration secondary to axonal disruption, there was an increase in phagocytic activity in these regions, although the phagocytic response was less pronounced than the MHC class II expression. These findings again focus interest on the white matter as a region of great importance in the long-term response to TBI. TUNEL positive cells, both oligodendrocytes [40] and macrophages/microglia [41], have been detected in the white matter of TBI cases many months after the injury. One interpretation of these findings is that axonal disruption may continue for many months after the initial forces associated with TBI have been applied, and that there is little, if any, axonal recovery; the neuroinflammatory response may contribute to or be secondary to this. Sulaiman *et al* [42], using the guinea pig stretch optic nerve model, showed on-going axonal and neuronal cell body degeneration over a 3-month period after a single stretch.

The distribution of tau pathology has been described in CTE [13], and after a single head injury with survival <12 months [17] and survival 1–47 years [18]. Tau pathology after TBI is described as involving predominantly superficial cortical layers, accumulating in the depths of sulci, and in a perivascular distribution. The distribution of activated microglia was diffuse and did not show any accentuation in these regions. This suggests that there is no direct spatial link between activated microglia and neurons expressing abnormal tau, although any potential interplay between activated microglia and neurodegenerative mechanisms that ultimately result in abnormal neuronal tau accumulation are likely to be much more complex and this observation in itself does not exclude a role for microglia in on-going TBI-related neurodegeneration.

Potential mechanisms and future studies

Microglia show considerable plasticity, and morphology may tell the investigator little about function [43]. Activated microglia may have a range of phenotypes, and these phenotypes are not fixed but will be modified by exposure to a changing local environment. Three broad macrophage phenotypes have been proposed; classically activated, wound healing and regulatory [44]. Microglia can bind amyloid fibrils via a number of receptors [45], although they are not effective at phagocytosing the fibrils, possibly as a result of co-expression of serum amyloid P (SAP) [46].

Activation of microglial cells can be produced by a variety of mechanisms. In a study of patients with a severe head injury IL-8 was noted to be markedly up-regulated acutely, with a x1000 increase in CSF when compared to peripheral blood levels [47]. The authors, therefore, postulated a role of this cytokine in initiating the neuroinflammatory response.

Cultured microglial cells have been shown to express $\alpha 7$ nicotinic acetylcholinergic receptors [48] and acetyl choline and nicotine pre-treatment can inhibit the lipopolysaccharide-induced microglial response. An $\alpha 7$ selective nicotinic antagonist can attenuate this inhibitory effect. Therefore, the intrinsic cholinergic system within the CNS may modulate the neuroinflammatory response. This system is frequently damaged in severe TBI and may result in loss of inhibition of the neuroinflammatory response [49]. A second receptor system which may modulate the microglial response is the purinergic receptor group P2X. Activation of the ionotropic P2X7 microglial receptor by extracellular ATP increases diacylglycerol lipase activity and inhibits monoacylglycerol lipase [50]. This may result in increased microglial production of 2-arachidonoylglycerol. This molecule is currently thought to have a major role in co-ordinating the neuroinflammatory response. 2-arachidonoylglycerol, via cannabinoid receptors, can reduce excitotoxic damage by reducing glutamate release [51], limiting cerebral oedema by reducing cerebral blood flow [52], and inhibiting the production of neurotoxic agents by microglia [53]. Recently modulation of interactions between the neuronal, glial and vascular compartments of the brain by adenosine has been studied in rodent models of neuroinflammation, with inhibition of the A_{2A} receptors (A2AR) limiting the neuroinflammatory response [54]. Clearly there are a number of potential molecular pathways that may be activated after TBI, and therefore a number of potential pharmacological targets to modulate the neuroinflammatory response. Our studies would indicate that any detrimental effect of neuroinflammation after TBI has a maximal effect around 3 months post-TBI, suggesting a time limited window for intervention if neuroinflammation is an important mechanism in long-term neurodegeneration.

-APP is not only up-regulated in acute TBI, but there is increased intraneuronal processing of the molecule [55] potentially resulting in A β production and deposition. Rapid A β deposition has been described in fatal head injury [56], and deposition of pathological A β is described in long-term survivors of a single episode of TBI [18]. It has been suggested that acute brain injury may damage synapses resulting in damage associated molecular pattern molecules (DAMPs) being released, producing a pro-inflammatory phenotype, whereas neuronal apoptosis may induce an anti-inflammatory phenotype [43].

Other potential functions of MHC class II up-regulation secondary to brain injury are speculative. Microglia are usually activated prior to astrocyte activation and gliosis although both cellular responses are commonly seen in response to brain injury. Microglia may act to control the astrocyte response and to limit the degree of gliosis [57] and may be involved in regulating synaptogenesis [58]. Microglia are important to the long-term re-organisation of neuronal synaptic connections after TBI. TBI results in primary neuronal loss (ischaemia, excitotoxicity) with substantial re-organisation of the residual tissue including synaptic sprouting and synaptogenesis. Eyupoglu *et al.* [58] studied microglial roles in synaptogenesis in both *in vivo* entorhinal cortex lesion and complex organotypic entorhino-hippocampal slice cultures. Pharmacological blocking of microglial activation protected neurons from microglia-induced secondary dendritic modification and promoted useful re-innervation. Synaptic degeneration is important in neurodegeneration, although whether this is primary or secondary pathology is unknown. Microglial activation and synaptic degeneration have been described in animal models [59] although a direct causative link between these two processes has not been firmly established.

As stated above, human neuropathological studies are limited by the human tissue available. In studies of TBI most cases are at the severe end of TBI and provide little information regarding the long-term effects of mild TBI. The development of ligands which bind to the peripheral benzodiazepine receptor on up-regulated microglia will allow the study of microglial activation state in living subjects. PK11195 labels activated microglia during life [60], and expression correlates inversely with cognitive function [61]. To date no studies have been undertaken in post-TBI patients, although a single paper studying a rat model of TBI demonstrated the effectiveness of both PK11195 and DAA1106, although suggested DAA1106 had a higher specificity [62]. Such ligands are now being used to study TBI and allow a direct comparison with the current post mortem based study [63]. These studies may guide future neuropathological work and may target different anatomical sites, such as subcortical areas.

Future studies will be required to assess the potential mechanisms whereby microglia may have deleterious effects on cognition. This may be by direct effects on neuron and axons resulting in increased neuronal and axonal damage, or may be more subtle, affecting synaptic organisation and structure. Our studies suggest microglial responses are maximal around 3 months, but that they return to control levels gradually over several years. It may be that by limiting microglial activation early on after traumatic brain injury, using agents such as nicotinic or adenosine antagonists, or cannabinoids, some TBI patients may have a better long-term outcome with regard to cognitive function.

In summary

- There was no significant increase in neuroinflammation with ageing in control cases.
- Increased microglial activity was seen after TBI, peaking around 3 months but returning to control levels after several years.
- There was increased expression of MHC class II and increased phagocytic activity in TAI cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CS, PDL and SMG undertook the assessment and interpretation. LSM undertook all statistical analyses.

Abbreviations

TBI	traumatic brain injury
TAI	traumatic axonal injury
AD	Alzheimer's disease

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Figure 1a:

Figure 1b:

Figure 1. Changes in the morphological appearances of microglia, in response to stimuli. Figure 1a shows ramified microglial cells (CR3/43 immunostaining x40), while figure 1b shows amoeboid (phagocytosing) microglial cells (CD 68 immunostaining x40).

Figure 2a- low load

Figure 2b- low load

Figure 2c- medium load

Figure 2d- medium load

Figure 2e- high load

Figure 2f- high load

Figure 2.

Examples of the variation of immunostaining seen between cases. The left column (figures 2a, c and e) was stained with CD68; the right column (figures 2b, d and f) was stained with CR3/43. All figures are $\times 10$.

Figure 3. Box and whisker plots of the overall immunoloads (CR3/43 and CD68) in the control group for those <20 years (n = 2), 20–49 years (n = 9) and 50+ years (n = 9)

Figure 4. Box and whisker plots of the overall immunolabel in the controls, those who survived less than one year and those who survived more than 1 year.

Figure 5.

Box and whisker plots of the overall immunolabel for differing survival intervals. The median immunolabel is emphasised with a large solid dot to illustrate the pattern over survival time.

Figure 6.

Box and whisker plots of the overall immunoload in those TBI cases who survived less than 1 year showing those with and without TAI.

Table 1

Details of cases used

Group	n	Age range (years)	Admission GCS range	Survival range
Control	20	18–71	NA	NA
Trauma age <20	15	1.5- 19	3–6	7 hours- 5 years
Trauma age 20–49	37	21–49	3–15	8 hours- 22 years
Trauma age >50	47	50–89	3–14	7 hours- 47 years

GCS= Glasgow Coma Scale

NA= Not Applicable

Table 2

TBI survival times in different age groups (n=99)

Age	0–19 years	20–49 years	50+ years
Survival			
<24 hours	4	4	5
1 day- 1 week	4	5	4
1 week- 1 month	5	4	4
1month- 1 year	1	5	12
1 year- 5 years	1	13	9
>5 years	0	6	13

Table 3

Details of control cases used in the neuroinflammatory study

	<20 years		20-50 years		>50 years	
	Age	Cause of death	Age	Cause of death	Age	Cause of death
Non-head injured controls with no neurological disease	18	Leukaemia	20	Drug overdose	50	Metastatic carcinoma
	18	Systemic Hodgkin's disease	21	Septic shock	55	Gastric lymphoma PTE
			24	Drug overdose	59	Disseminated Langerhan's histiocytosis
			28	Drug overdose	60	Bronchial carcinoma
			33	Ischaemic heart disease	64	Sarcoidosis
			35	Malignant teratoma	69	Acute pyelonephritis
			43	Hodgkin's lymphoma	69	Congestive cardiac failure
			44	Myocardial infarct	70	Breast carcinoma
			47	T cell lymphoma	71	Pneumonia

PTE= pulmonary thrombo-embolus