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Recent Advances in Neurotrauma

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Abstract. The frequency of and outcome from acute traumatic brain injury (TBI) in humans are detailed together with a classification of the principal focal and diffuse pathologies, and their mechanisms in extract laboratory models are outlined. Particular emphasis is given to diffuse axonal injury, which is a major determinant of outcome. Cellular and molecular cascades triggered by injury are described with reference to the induction of axolemmal and cytoskeletal abnormalities, necrotic and apoptotic cell death, the role of Ca^{2+}, cytokines and free radicals, and damage to DNA. It is concluded that TBI in humans is heterogeneous, reflecting various pathologies in differing proportions in patients whose genetic background (*APOE* gene polymorphisms) contributes to the outcome at 6 months. Although considerable progress has been made in the understanding of TBI, much remains to be determined. However, a deeper understanding of the pathophysiological events may lead to the possibility of improving outcome from rational targeted therapy.

Key Words: ApoE genotype; Apoptosis; Outcome; Traumatic brain injury.

INTRODUCTION

The pathology of head injury is complex, the outcome being a product of different mechanisms, types, and amounts of brain injury and their anatomical location. Epidemiological studies have shown that deaths from head injury comprise 1%–2% of all deaths from all causes, and that between one third to one half of all traumatic deaths are due to head injury. The precise incidence of head injury is difficult to establish. In the UK about 400 patients per 100,000 of the population are admitted to the hospital each year with head injury, of which 30 per 100,000 are adults not obeying commands. In a recent survey of over 1,000 patients admitted to hospital after head injury, the European Brain Injury Consortium found 31% were dead in 6 months, 30% had made a good recovery, 20% were moderately disabled, 16% were severely disabled, and 3% were vegetative (1).

Classification of Brain Damage

There are 2 main categories of damage after a head injury: 1) primary, that occurs or is triggered at the moment of injury (lacerations of the scalp, fracture of the skull, surface contusions and lacerations of the brain, diffuse axonal injury and intracranial hemorrhage); and 2) secondary, produced by complicating processes that are initiated at the moment of injury but do not present clinically for a period of time after injury (damage due to raised intracranial pressure, ischemia, swelling and infection) (2). Neuroimaging as a means of identifying intracranial pathology after head injury has allowed the clinical adoption of an alternative classification of focal and diffuse damage. Experience has shown that whereas the nature of a focal lesion can be determined in life with a high degree of certainty, it is more difficult in comatose cases with diffuse brain damage who do not demonstrate an intracranial mass lesion.

Mechanisms of Brain Damage

These include static loading and the various types of dynamic loading (2). Static loading occurs when forces are applied to the head gradually and a slow time course (usually taking more than 200 milliseconds [ms] to develop). Sufficient force may cause multiple, comminuted, or eggshell fractures of the skull, and may not be associated with either coma or severe neurological signs until the amount of skull deformation is so severe that the brain itself becomes compressed and distorted. The most common mechanical input to the head is dynamic loading of either impact or impulsive type (act in less than 200 ms, and, in most instances, in less than 20 ms). Impact loading occurs when a blunt object strikes the head and usually initiates a combination of contact and inertial forces that result in a series of events that vary with the size of the impacting object and the magnitude of the force delivered to the contact point. In addition, shock waves are propagated throughout the brain. Impulsive dynamic loading occurs when the head is set into motion or when the moving head is stopped without it striking anything or is arrested by impact. Under these conditions the resulting head injuries are caused solely by the inertia produced by the manner (translation, rotation, angulation) in which the head is moved. Strain is the proximate cause of tissue injury whether induced by contact or inertia. Characteristically, biological tissues will stand the strain better if they are deformed slowly rather than quickly, i.e. they become more brittle and will break at lower strain levels under rapidly applied loads.

There is now a wealth of information that lesions due to contact include lacerations of the scalp, fracture of the skull

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with or without an associated extradural (epidural) hematoma, surface contusions and lacerations, and intracerebral hematoma. In contrast, inertial forces are responsible for the 2 most important types of damage encountered in blunt head injury, namely, acute subdural hematoma resulting from tearing of subdural bridging veins and widespread damage to white matter in the form of diffuse axonal injury. Analysis of various data sets (2) has shown that focal damage associated with contact is more likely to be sustained as the result of a fall, while diffuse damage is most commonly associated with acceleration/deceleration occurring after, for example, traffic accidents.

Brain damage after head injury in humans is heterogeneous. For example, in a human clinical series, solitary lesions were found in 26% of cases while 2–3 or more than 3 lesions occurred in 21% and 27% of cases, respectively. In a comprehensive neuropathological study of 50 cases from the Institute of Neurological Sciences, Glasgow, focal lesions were found in 47 cases (95%) and diffuse lesions in 48 (94%) of cases. Overall there were only 4 cases (8%) in which there was a single type of lesion, 6% in which there were 2, and 14% in which there were 3 pathologies. But in over 72% of the cases there were 4 or more types of lesion, providing further evidence that as injury severity worsens so the multiplicity of lesions increases.

Experimental Models of Human Blunt Traumatic Brain Injury-Matching Models to Humans

The principal models of experimental head injury and the major pathological findings in them have been comprehensively reviewed (3) and the specific attributes of each model in terms of its ability to reproduce abnormalities known to occur in human head injury have been detailed. Replication of diffuse brain injury has been achieved by angular acceleration in nonhuman primates (4), in the mini-pig (5), and by contact percussion in rats (6). Typically there is widespread microscopic evidence of damage to axons (7). Contusion is easy to replicate and can be induced by suction and local tensile models without the complications of more distant or global changes: there is a hemorrhagic focus of necrosis, disruption of the blood-brain barrier (BBB), the formation of vasogenic edema, decreases in regional cerebral blood flow, and an increase in glucose metabolism. Histologically, neutrophil polymorphs increase in number by 24 h followed by activation of microglia (8) and the formation of macrophages; gliosis develops and a glial limitans is formed to separate injured from intact neuropil. A more difficult entity to model is that of acute subdural hematoma, although this can be achieved by injection and acceleration (3); however, the associated features of skull fracture, brain swelling, and extradural hematoma are not easily duplicated routinely.

To date, experimental models have contributed greatly to our insight of post-traumatic mechanisms of cell death and behavioral disability. Additionally, they have been integral in the development of several novel diagnostic and/or treatment strategies that have either become part of standard clinical practice or are now under preclinical or clinical investigation.

All models designed to produce experimental TBI should fulfill several criteria: these include the production of a quantifiable and reproducible injury, and the employment of standardized surgical protocols and techniques. The latter should include the use of ''sham'' (uninjured) animals to control for surgical/systemic variables (e.g. the operative procedure, anesthesia, or alterations in core or brain temperature), and any brain damage due to head restraint. The majority of devices inducing trauma currently use computer-based measurements of the applied load (e.g. pressure gradients, velocity of impactor, amount of acceleration/deceleration) to assess for variation of the mechanical parameters that define injury severity. This information is critical in making adjustments to the injury device, permitting the maintenance of a narrow range of injury severity within a given study, and the generation of a reproducible injury between experimental animals.

The fluid percussion model of experimental brain injury is the most commonly used and well-characterized model of clinical traumatic brain injury. This model, which was originally developed for the rabbit and cat, has been modified over the past decade to produce injury in a wide range of differing animal species including mouse, rat, dog, and pig (2, 3). Fluid percussion reproduces contusions as focal grey matter damage with accompanying intraparenchymal or subarachnoid hemorrhage. Central or midline fluid percussion in rodents leads to contusion directly at the site of fluid impact and compression of brainstem structures with resulting behavioral disturbances resembling coma when more severe levels of injury are induced. The lateral fluid percussion model in the rat produces a lesion cavity that is associated with both necrotic and apoptotic cell death, evolves from the contusion over a period of days to weeks, and then progressively expands for up to one year post-injury (9, 10). Progressive changes in the hippocampi and thalami have also been reported in this model, features that have been accompanied by persistent cognitive (11) and motor impairment up to one-year post-injury (12). Although the lateral fluid percussion model was developed as a model of unilateral damage, a number of studies have shown that the damage is widely distributed.

The plethora of models designed to study brain injury further emphasizes the complexity and heterogeneity of TBI in humans. In particular, it should be remembered that with few exceptions human head injury is rarely pure, in contrast to many of the experimental models that

serve to isolate different aspects of traumatic brain injury. Thus, using the purer and extract experimental models, it may not be possible to adequately address the multiplicity of focal and diffuse pathologies seen in many patients with traumatic brain injury.

THE IMPORTANCE OF DIFFUSE BRAIN DAMAGE INCLUDING DIFFUSE AXONAL INJURY

The descriptor ''diffuse brain injury'' refers to the phenomenon of widespread damage to either grey or white matter (or both) rather than to a localized area of injury—focal injury—where the remainder of the brain is, apparently, unaffected. The pathological manifestation of diffuse brain injury will differ with the type of insult that the brain has received. The principal types of diffuse brain damage are brain swelling, hypoxic damage, and diffuse axonal injury (DAI), of which the latter is particularly important.

Various categories of diffuse white matter injury are now recognized as having a similar pathophysiology, but there are major differences in the clinical outcome of such injury, varying with the severity of the mechanical strain placed upon axons. At the least severe level of injury are the concussion syndromes in which there is a short-term loss of neurological function but not of consciousness. A greater severity of injury results in ''classical cerebral concussion'' in which there is loss of consciousness for a period of usually less than 6 h. At the most severe level there is prolonged traumatic coma that is not associated with an intracranial mass lesion but in which there is diffuse axonal injury (2, 13). Patients with severe DAI are unconscious from the moment of injury, do not experience a lucid interval, and remain unconscious, vegetative (14), or at least severely disabled until they die, usually as a result of secondary, post-traumatic complications.

In the head-injured patient, a definitive diagnosis of DAI can only be made postmortem since the principal criterion for its diagnosis is the presence of injured axons. Techniques for the demonstration of injured axons in brain have undergone considerable development in recent years. Initially, silver impregnation techniques for the labeling of axonal ''retraction'' bulbs were used. More recently, a number of immunocytochemical (ICC) methods have been introduced with the general acceptance that greatest sensitivity of labeling, both for postmortem diagnosis and in the experimental situation of damaged axons, may be obtained using ICC labeling for β-amyloid precursor protein (β-APP) (15, 16). This technique is now widely used to identify injured axons scattered among a population of uninjured ones, and has provided the added benefit that damaged axons may be demonstrated within 2–3 h of injury (15, 16). Further, the technique has allowed discrimination of changes in axonal form prior to axotomy in that focal ''axonal swellings'' (7) precede the occurrence of ''retraction bulbs.'' Labeling for β-APP has also allowed demonstration of injured axons in humans up to 99 days after mild head injury (17).

Concept of Traumatic Axonal Injury

As originally described, DAI was defined as a focal lesion in the corpus callosum, focal lesions in one or both dorsolateral sectors of the rostral brainstem adjacent to the cerebellar peduncles, and diffuse damage to axons (2). Such a distribution of injured axons has been achieved in only one animal model, the nonhuman primate (4).

Recently, the descriptor traumatic axonal injury (TAI) has been applied to experimental studies in animals that have attempted to elucidate the mechanisms of axonal pathology after trauma. In TAI, injured axons form focal swellings at intervals along their length within the first 2 h after injury. These swellings increase in size until the axon undergoes disconnection between 4 and 6 h after injury; a process now termed secondary axotomy (7). This time course parallels that found in DAI but the current level of understanding of the events in injured axons leading to secondary axotomy has been derived from studies of experimental traumatic axonal injury.

Strich was the first to define ''diffuse degeneration of the cerebral white matter'' where nerve fibers were sheared or mechanically torn apart at the time of injury (18). Good evidence in support of this concept has been obtained in only one animal model (7). The concept has recently arisen that axons are not sheared at the time of injury, except perhaps in the most severe form of DAI, but rather they are damaged and enter a ''pathological cascade'' of events leading to axotomy at least several hours after the initial injury. Where nerve fibers are sheared at the time of injury (primary axotomy), the axolemma is fragmented and there is rapid loss of the axonal cytoskeleton such that neurofilaments and microtubules are replaced by a flocculent ultrastructure suggestive of autolysis (7). However, where the axolemma has been fragmented there is not a loss of membrane specializations such as the dense undercoating characteristic of the node of Ranvier.

In the last several years a consensus has arisen that the prime site of injury is the axolemma and that pathology of the injured axon occurs because of loss of homeostatic mechanisms in the maintenance of the differential ionic gradients necessary for the electrical activity of the axon. Evidence for damage to the axolemma has been obtained through use of several experimental techniques. First, the electron dense tracer horseradish peroxidase (HRP) is normally excluded from the axoplasm when the tracer is injected into the CSF. However, upon TAI, peroxidase floods the axoplasm of injured nerve fibers. The precise mechanism of entry for HRP is unresolved but it has been suggested that the axolemma becomes leaky or is perturbed (19, 20). Second, quantitative analysis of freeze-fracture replicas (21) and cytochemical localization of

certain axolemma membrane pumps has demonstrated changes in the structure of the axolemma in injured nerve fibers and alteration in the localization of Ca^{2+} -ATPase and p-NPPase activity after TAI (7, 22). These studies provide strong support for the hypothesis that after TAI there is an uncontrolled influx of Ca^{2+} into injured nerve fibers, a morpho-pathological manifestation of which is swelling of axonal mitochondria (22). Elevated intra-axonal levels of Ca^{2+} are currently hypothesized to result in depolymerization of microtubules, as reflected in the rapid acute reduction in their number (7), activation of calpains that lead to alterations in axonal inter-neurofilament relationships, and changes in axonal transport that allow the development of axonal swellings and potentiate secondary axotomy. Recent ICC demonstrating spectrin breakdown products in injured axons has served also to greatly strengthen this hypothesis (23).

Early in the 1990s it was hypothesized that the application of transient tensile strain to axons directly disrupts the relationships between components of the axonal cytoskeleton after traumatic brain injury. This has now been superseded by the view that injury to the axolemma is the principal cause of the ensuing axonal pathology. Where swollen axons are labeled for β-APP, it is hypothesized that there is a focal disruption of axonal transport. Support for this hypothesis has been provided by quantitative analyses of changes in the axonal cytoskeleton after TAI, after which there is a dramatic loss of the number of axonal microtubules (25) and a reduced spacing, termed compaction, between neurofilaments within injured axons (19, 24). Loss of microtubules has been hypothesized to result from their depolymerization when intra-axonal Ca^{2+} levels are elevated post-trauma (7). Loss of microtubules will clearly result in loss of fast axonal transport and allow the accumulation of both β-APP and membranous organelles at foci that then form axonal swellings. The significance of compaction of neurofilaments is presently unclear, except in that slow axonal transport will, probably, be compromised. However, compaction of neurofilaments has been noted within minutes of TAI, and neurofilaments remain in a compacted state for at least several hours prior to the axon undergoing secondary axotomy. At the point of axotomy there is loss of integrity of the axolemma and dissolution of the compacted neurofilaments (24). It is also clear now that degeneration of the axonal cytoskeleton is a result of damage to the axolemma rather than a direct effect of mechanical trauma.

CURRENT CONCEPTS OF THE PATHOPHYSIOLOGY OF TRAUMATIC BRAIN INJURY

Initial Events

The biomechanics of ''primary'' or mechanical damage are linked to the response of bone, blood vessels, and the brain to both impact and inertial forces. With direct impact to the skull, a local bending occurs with underlying tissue strain and gross movement of brain. Conversely, inertial injury does not create local contact effects but produces a nonuniform distribution of pressure and tissue strain that cause primary tissue damage (2). Intracranial pressure changes and brain motion due to translational acceleration have been linked to specific focal lesions such as coup and contrecoup contusions, intracerebral and/or subdural hematomas, and brainstem lesions. Other lesions, such as DAI and gliding contusions, are more related to rotational acceleration forces (2).

Secondary Events

Superimposed on trauma-induced mechanical injury, ''secondary'' or delayed neuronal or cellular damage develops over a period of hours, days, or weeks after the initial trauma. This type of damage appears to be associated with trauma-induced neurochemical alterations, which can exert either direct pathogenic effects on regional cerebral blood flow, BBB function, cerebral metabolism, and ion homeostasis, or have direct neurotoxic effects on regional populations of neurons or glial cells (26). These post-traumatic cellular and molecular changes may involve alterations in the synthesis and release of both neuroprotective and autodestructive or neurotoxic cascades (27). Identification of these pathways will provide important information about mechanisms of damage and stimulate the development of novel therapeutic strategies designed to prevent or attenuate trauma-induced damage to the CNS. In this review, we have chosen to detail selected secondary injury pathways that have been currently and most convincingly related to the pathobiology of neuronal death associated with traumatic brain injury.

Is ''Cytoskeletal Collapse'' a Major Feature of the Response to Traumatic Brain Injury?

Alterations in brain Ca^{2+} homeostasis (13, 22, 28) and receptors/channels associated with Ca^{2+} entry (voltage sensitive channels or ionophore-associated glutamate receptors such as N-methyl-D-aspartate-NMDA receptors) have been associated with regional cerebral edema, vasospasm, and delayed cell death. Traumatic, ischemic, or anoxic injury to neurons is associated with widespread neuronal depolarization (including the induction of cortical spreading depression) and release of excitatory amino acid neurotransmitters such as glutamate, leading to the opening of NMDA receptor-associated ion channels and influx of Ca^{2+}. The post-traumatic Ca^{2+}storm has been documented using ^{45}Ca-autoradiography (29) and indirectly via cytochemical evidence for redistribution of membrane pump calcium-ATPase and ecto- Ca^{2+}-ATPase activity, and Ca^{2+} influx in myelinated nerve fibers of the guinea-pig optic nerve after stretch (22) and the analysis

of calcium-mediated gene expression (28). Both Ca^{2+}-channel blockers and competitive and noncompetitive NMDA receptor antagonists have been shown to be efficacious in the treatment of experimental TBI (27), but to date have been disappointing in human studies (30).

Pathological elevations in intracellular Ca^{2+} after TBI can precipitate an attack on the lipid bilayer cell membrane via the activation of calcium-dependent phospholipases and generation of reactive oxygen species (26, 27). These highly reactive molecules cause direct peroxidative destruction of the cell membrane, oxidize cellular proteins and nucleic acids, and destroy the cerebral vasculature. Calcium can also activate nonlysosomal cysteine protease calpain, which can degrade a wide range of cytoskeletal protein substrates, including spectrin (23), tubulin, microtubule-associated proteins MAP-1B, MAP-2, and the neurofilament protein family. The activation of calpain can be determined by direct detection of autolyzed calpain or indirectly via detection of calpain-specific proteolytic fragments. Several recent studies have documented both acute calpain activation and regional calpain-induced cytoskeletal proteolysis (23, 31, 32). Both the neuronal and axonal cytoskeleton appears to be vulnerable to calpain-induced proteolysis (7, 33) in laboratory models and in human (34), and therapeutic strategies to block or antagonize the proteolytic effects of calpain on the cytoarchitecture of the cell have proven effective (35) by attenuating both post-traumatic motor and cognitive deficits (31).

Is Inflammation a Major Feature of the Response to TBI?

There remains little doubt that a role exists for post-injury inflammation in mediating delayed neuronal damage. Alterations in blood-borne immunocompetent cells have been described after trauma, and since the BBB is opened during the acute post-traumatic period, entry into the brain of these cells may directly influence neuronal death and/or survival. Infiltration and accumulation of polymorphonuclear leukocytes (PMNs) into brain parenchyma has been documented in experimental TBI in rats (26) and, in the first 3 days after human TBI (2, 36). The entry of PMNs into injured brain and activation of microglia/macrophages is believed to be both pathogenic in mediating the local inflammatory response, and a participant in reparative and/or regenerative processes. However, the role of the marked increase in regional concentrations of the cytokines interleukin-1 (IL-1B), interleukin-6 (IL-6), and tumor necrosis factor (TNF) observed after experimental TBI remains uncertain. Recent studies documenting the beneficial effects of pharmacological blockade of the complement cascade and the cytokines IL-1B (37, 38) and TNF (39) suggest that the release and/or upregulation of these pathways may be indeed pathogenic. However, more recent work with the transgenic mouse tumor necrosis factor (TNF/Lymphotoxin-d (LT-α) and interleukin 6 (IL-6) and in wild-type litter mates subjected to experimental closed TBI has shown that in spite of an increased post-traumatic mortality in TNF/LT-α-deficient mice, there is a neuroprotective effect of these cytokines (40). The results suggest that these cytokines may play a role in facilitating long-term behavioral recovery, thereby underscoring the potential for post-injury cellular and molecular changes to be either pathological or protective, depending on when they are expressed during the post-injury cascade.

Is There Neurodegeneration after Traumatic Brain Injury?

It is well established that TBI is a major epidemiological risk factor for Alzheimer disease (AD) (see below) and a pathological hallmark of this disease (diffuse β-amyloid deposits) has been reported to occur between 4 h and 2.5 yr in human head-injured patients (41). Attempts to reproduce these clinical observations using experimental models of TBI in rodents have largely failed with exception of a diffuse increased expression of β-APP, although neurofilament inclusions in a pig model of diffuse TBI have been reported (42). Recent studies employing transgenic mice engineered to over-express human APP 2-fold (APP-YAC mice) failed to produce amyloid plaques or altered behavioral outcome (43). A subsequent study with transgenic mice over-expressing human APP 10-fold (PD-APP mice) reported evidence of exacerbated hippocampal cell loss, significantly increased regional concentrations of Aβ-PP 1–42, and worsened cognitive function after traumatic brain injury (44). TBI in young PD-APP mice has also been shown to induce marked ipsilateral hippocampal atrophy with diminished Aβ-PP deposition during aging (45), suggesting that the vulnerability of brain to Aβ-PP toxicity increases while the accumulation of Aβ-PP deposits decreases over a period of months after traumatic brain injury.

The brain damage associated with dementia pugilistica (see below) and its associated memory loss, Parkinsonian-like tremors and gait, underscores the potential relationship between TBI and other neurodegenerative diseases such as Parkinson disease. Because increasing evidence suggests that neurofilament-rich inclusions have deleterious effects on neuronal function and survival, transgenic mice expressing excessive levels of heavy-chain neurofilament protein fused to a beta-galactosidase reporter gene (NFH-LacZ mice) were subjected to experimental TBI and found to be more behaviorally and histologically vulnerable to TBI than wild type mice (46). These data suggest that the presence of NF-rich inclusions may exacerbate neuromotor dysfunction and cell death after traumatic brain injury.

Does the Response to TBI Recapitulate Developmental Changes?

While necrotic cell death has been extensively documented after both clinical and experimental TBI, it has been suggested there is also an induction of neuro-developmental cascades including programmed cell death (PCD). Unlike necrosis, PCD involves the initiation and active expression of transcription and translation-dependent pathways in which apoptosis is regarded as the primary hallmark. Apoptosis has been classically associated with the formation of the normal CNS during development and, although necrosis and apoptosis have been more traditionally considered as distinct mechanisms, it may be possible to consider them to be part of the same continuum of cell death, particularly within the context of traumatic CNS injury. Using a combination of terminal deoxynucleotidyl transferase (TdT)-mediated biotinylated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) histochemistry with electron microscopy and DNA gel electrophoresis, it has been possible to identify apoptotic cells after experimental lateral fluid percussion brain injury in the rat (47). These observations have been extended by demonstrating regional and temporal differences in apoptotic cell death cascades, in astrocytes, oligodendrocytes, and neurons some months after experimental traumatic brain injury in a number of TBI models (48–50), and by the presence of apoptotic, TUNEL-positive neurons and oligodendrocytes in the tissue of surface contusions of human head-injured patients (51).

The Bcl-2 superfamily of cell death regulatory genes, originally associated with developmental cell death, has been implicated as a mediator of post-traumatic apoptosis. Increased expression of the anti-apoptotic protein Bcl-2 has been observed in surviving neurons after both experimental and clinical traumatic brain injury (52, 53), while Raghupathi et al (personal communication) have found an acute downregulation of bcl-2 in hippocampal and cortical neurons destined to die after experimental CNS injury. Transgenic mice over-expressing human Bcl-2 protein exhibit significantly less neuronal loss in the injured cortex and hippocampus (54). Bcl-2 proteins may also participate in the control of cell death and survival by regulating the release of cytochrome c from mitochondria, which itself participates in the activation of members of the caspase family of death-related proteases. This gene family contains up to 12 known members that, during CNS development, are associated with the final steps in the apoptotic cascade. Specific caspases, including caspase-3, may cleave substrates associated with DNA damage and repair, including DNA-fragmentation factor (DFF45/40), poly(ADP-ribose), polymerase (PARP), and cytoskeletal proteins actin and laminin. It has been reported recently that caspase-associated cleavage products of all of these substrates are expressed after experimental traumatic brain injury (55; Barey, personal communication). Caspase-3 itself has been also shown to be activated in injured cortex in the acute period after experimental (49, 56) and human (53) brain injury, and a reduction in post-traumatic apoptosis and neurological deficits have been reported after central administration of the caspase inhibitor z-DEVD-fmk after lateral fluid percussion brain injury in rats (49).

In the CNS, neurotrophic molecules have a profound influence on the development and maintenance of neuronal innervation, differentiation, and process outgrowth. For example, nerve growth factor (NGF) plays a major role during normal development in supporting neurons that have made appropriate connections, while basic fibroblast growth factor (bFGF) has trophic effects in CNS development and promotes neurite outgrowth and astrocyte/oligodendroglial proliferation. Several recent studies suggest that tissue content of growth factors is altered after TBI: both NGF gene and protein expression have been shown to markedly increase in the acute post-traumatic period in rats (57) and after mechanical injury to organotypic hippocampal slices in vitro (58). It has been hypothesized that the upregulation of NGF after TBI serves as a mediator of oxidative homeostasis by inducing the production of free radical scavengers such as glutathione (59). Although the bFGF response has not been evaluated in models of TBI, concentrations of both NGF and bFGF in the CSF of head-injured patients have been reported to be increased (60). Hippocampal expression of brain-derived neurotrophic factor (BDNF) and RNA has been shown to increase bilaterally by 3 h after lateral fluid percussion brain injury (61).

In addition to the injury-induced response of trophic factors, convergent evidence suggests that an upregulation of several growth-related proteins such as growth-associated protein 43, MAP1B, and polysialylated neural-cell adhesion molecule (PSA-NCAM) occurs after brain injury (62, 63), providing further support for a ''recapitulation'' of early CNS developmental events. It is possible that this developmentally appropriate injury response represents an attempt at regeneration by the adult CNS to traumatic injury.

Does the Response to TBI Mimic Oncogenesis?

The association between TBI and apoptotic cell death and alterations in cell death/survival pathways including the *Bcl-2* family of genes have been reviewed (52, 53). Other parallels such as single- or double-strand DNA breaks associated with apoptotic cell death and oncogenesis have also been described (49, 50, 54). Activation of endonucleases can result in double-strand DNA breaks, whereas single-strand breaks are often associated with oxidative damage and formation of reactive oxygen species (ROS). These ROSs, such as the superoxide anion,

the hydroxyl radical, or singlet oxygen have all been associated with traumatic CNS injury. In addition to causing DNA strand breaks, ROS can mediate the formation of DNA-protein adducts and/or oxidative adducts of the nitrogen bases, which can participate in DNA fragmentation.

Damage to intracellular DNA may lead to apoptosis, arrest of the cell cycle, or cell growth and/or elimination/repair of the damaged DNA. The tumor suppressor gene *p53*, often termed the ''guardian of the genome,'' has been shown to be induced and upregulated in response to DNA damage. Induction of this gene has been reported after both fluid percussion and controlled cortical impact brain injury in the rat, where increased p53 mRNA and protein were observed in regions exhibiting TUNEL-positive apoptotic cells (64, 65). Under conditions associated with cell cycle arrest, p53 has been suggested to mediate the induction of cyclin D1, a regulator of cyclin-dependent kinases that is essential for progression through the G1 phase of the cell cycle. However alterations in mRNA for cyclin D1 were not observed in the acute period after TBI (65).

Base excision repair (BER) and nucleotide excision repair (NER) are the primary mechanisms responsible for the repair of damaged DNA. The BER pathway involves activation of specific repair enzymes, including glycosylases, endonucleases, and ligases and has been associated with oxidative DNA damage. The NER pathway involves the activation of both exo- and endonucleases to remove the patch of injured or mutated DNA strand that is subsequently replaced through the activation of DNA polymerases. It has been proposed that during oncogenesis and tumor formation, these repair mechanisms are compromised. Interestingly, a decrease in nuclear endonuclease activity associated with BER pathways has been reported after experimental global cerebral ischemia, suggesting that neuronal apoptosis may occur from a failure of DNA repair (66). Moreover, poly(ADP ribose) polymerase (PARP), a nuclear protein that acts as an endogenous detection system for DNA damage repaired by BER mechanisms (including both single- and double-strand DNA breaks) has been shown to be activated in the acute post-traumatic period after lateral fluid percussion brain injury (55). Subsequent inhibition of PARP activation in the later stages of TBI in this same study may be related to caspase-3 induced proteolysis associated with apoptosis and impaired repair of damaged DNA.

Recently, a heterodimeric protein composed of 40- and 45-kDA subunits, designated DNA fragmentation factor (DFF), which is required for the initiation of endonuclease-mediated DNA fragmentation when DFF45 is cleaved by caspase-3, has been identified. Activated caspase-3 will dissociate DFF45 from DFF40 (the active component of DFF), which then triggers both DNA fragmentation and chromatin condensation during apoptosis. Although these events are believed to play a role in apoptosis associated with oncogenesis, it has recently been demonstrated that caspase-3-associated cleavage of DFF45/DFF40 occurs after lateral fluid percussion brain injury (67), which may also play a role in acute neuronal death. The relationship between cell death pathways associated with oncogenesis and those activated by TBI remain intriguing and warrant further investigation.

LONG TERM SEQUELAE OF TBI AND THE INFLUENCE OF GENETIC FACTORS

Long-Term Outcome after TBI

Long-term follow-up studies after head injury document persisting problems even after relatively mild injury. There is relatively little direct high quality information concerning the outcome after many years or decades post-injury. However, studies from several different perspectives point to the occurrence of late neurodegenerative events many years after injury (68), although comprehensive neuropathological studies have not been undertaken.

There has been much interest in the long-term outcome in sporting participants who have experienced relatively minor but repeated head injury. Most obviously these include boxers, a proportion of whom develop a late cognitive decline (dementia pugilistica) often with Parkinsonian features (69). The neuropathology comprises a loss of neurons in the substantia nigra and some features in common with AD, including neurofibrillary tangles and Aβ-PP containing plaques (70). Of recent interest has been the demonstration of neurofibrillary tangles in the cerebral cortex of young adults in their twenties who had sustained relatively minor repeated head injury due to boxing and other activities (71).

Further information on the long-term effect of head injury has come from a meta-analysis of studies performed in the 1980s that showed a significant association between a history of head injury with loss of consciousness and the risk of subsequent AD, a finding confirmed by subsequent studies (72). Among patients who developed AD and who had a head injury in the past, the age of onset of which was 8 yr less than among patients who had developed AD without a history of head injury (73).

Evidence For a Genetically Determined Influence on Outcome after Traumatic Brain Injury

Exploration of the apparent association between head injury and the subsequent development of dementia revealed a potential mechanism to explain the link. Plaque-like deposits of Aβ-PP (Aβ) were found in the cerebral cortex of approximately 30% of patients dying in the acute stage after head injury: deposits of Aβ-PP occurred

in a higher proportion of head-injured patients than in age-matched controls (41). From these findings it was inferred that head injury can trigger the deposition of Aβ in the cerebral cortex and that this, in keeping with the ''amyloid hypothesis'' of AD, could render survivors vulnerable to later neurodegenerative processes. After the recognition that possession of the ϵ4 allele of the apolipoprotein E (*APOE*) gene is a major genetic risk factor for sporadic AD, the relation of the *APOE* gene polymorphism to the Aβ-PP deposits in fatal head injury was explored (74). Patients with *APOE* ϵ4 who died from head injury were more than 4 times as likely to have cortical Aβ-PP deposits than patients without *APOE* ϵ4. In view of the previous studies this was interpreted to indicate that head injury can act as a trigger for Aβ-PP deposition in those people, about a third of the population, with a genetic susceptibility conferred by *APOE* ϵ4. This evidence suggesting that the neuropathology of head injury may be influenced by the *APOE* gene polymorphism was a prelude to clinical studies of outcome.

Several studies have now shown that patients with *APOE* ϵ4 have a worse outcome after traumatic brain injury. A small study (n = 16) of patients with prolonged post-traumatic coma revealed a higher frequency of *APOE* ϵ4 among patients who did not recover consciousness than those who did (75). In a prospectively recruited series of patients admitted after a head injury to a neurosurgical unit (n = 93), it was found that 57% of patients with *APOE* ϵ4 had an unfavorable outcome (dead, vegetative state, or severe disability according to the Glasgow Outcome Scale) 6 months after injury, compared with 27% of the patients without *APOE* ϵ4 (76). This remained significant when controlling for age, the severity of the initial injury as assessed by the Glasgow Coma Score (GCS), and initial CT scan findings. However, it is worthy of note that a higher proportion of the patients with *APOE* ϵ4 had a low initial GCS, raising the possibility that their poorer response to the injury may be manifest within hours. A subsequent study found that *APOE* ϵ4 is strongly associated with more than 7 days of post-traumatic unconsciousness and patients with *APOE* ϵ4 are unlikely to have a good functional outcome (77). It has also been shown that *APOE* genotype influences outcome after traumatic brain injury associated with boxing. A study of 30 professional boxers (aged 23–76 yr) showed that high exposure boxers (i.e. those with 12 professional bouts or more) who carried *APOE* ϵ4 had significantly greater scores on a clinical scale of chronic traumatic brain injury (78).

Studies addressing the influence of *APOE* genotype on the longer-term outcome after trauma, and particularly whether *APOE* ϵ4 predisposes to a late neurodegenerative decline, are awaited. However, of note is the evidence that there is a synergistic interaction between a history of a head injury and possession of the *APOE* ϵ4 allele as risk factors for AD (79).

Potential Underlying Pathological Mechanisms

It is tempting to focus on the deposition of Aβ-PP as the pathological event triggered by head injury and predisposed to by possession of *APOE* ϵ4. Other studies implicate apoE in binding Aβ-PP and promoting aggregation of fibrils, and *APOE* ϵ4 shows a robust association, in AD and in aging, with Aβ-PP deposition both in plaques in the cerebral cortex and in blood vessels as cerebral amyloid angiopathy. Particularly with the knowledge that Aβ-PP deposition is an early event in AD, and by analogy with dementia pugilistica, the possibility was raised that head injury related Aβ-PP deposition in those who survive may be followed by the development of AD later in life (41, 74). However, the original interpretation that head injury can trigger the acute deposition of Aβ-PP has been questioned and the suggestion raised that the Aβ-PP deposits could predate the head injury. It was proposed that individuals with *APOE* ϵ4 were more likely to have pre-existing Aβ-PP deposits at a young age and that individuals with *APOE* ϵ4 may be more likely to die after their injury, therefore permitting their inclusion in a post-mortem study (80). This issue has not been resolved and it may not be possible to do so within the constraints of studies of human autopsy tissue. However, it is of interest from animal models that plaque-like deposits of Aβ-PP can indeed form within days (81, 82). This experimental finding supports the hypothesis that Aβ deposition in humans could be triggered by head injury and the possibility, in survivors of head injury with *APOE* ϵ4, that this could predispose to AD-like neurodegeneration later in life.

However, exploration of other potential mechanisms that may mediate the influence of *APOE* genotype is warranted. Indeed, it is remarkable the number of processes in which apoE appears to be involved, both in the CNS and the circulatory system which supports it. For example, of potential relevance to the acute response to injury is evidence that apoE is involved in the maintenance of cytoskeletal integrity, protection from oxidative stress, and modulation of the response to excitotoxicity. *APOE* ϵ4 predisposes to atherosclerosis, ischemic heart disease, and cerebral amyloid angiopathy, which could compromise cardiovascular integrity in patients with injuries resulting from trauma. Of potential relevance to longer-term outcome apoE is involved in the delivery of lipids to neurons required for neurite outgrowth and synaptogenesis (83), clearance of degeneration products, microglial activation, and in maintenance of the cholinergic system (84).

As yet it is unclear which specific mechanism or mechanisms in which apoE is involved are responsible for the poorer outcome in head-injured patients with *APOE* ϵ4.

However, both human and animal neuropathological studies have highlighted the importance of apoE in the acute response of brain injury. After brain injury there are marked alterations in the cellular localization of apoE, most notably with an increase in neuronal immunoreactivity (85, 86). Studies of genetically modified mice have provided further evidence that apoE modulates the response to injury. For example, apoE deficient mice have more severe lesions than wild type mice (86), and mice bearing the human ϵ4 allele have more severe lesions than those with ϵ3 (87). ApoE appears to have a direct neuroprotective role since neuronal damage in *APOE*-deficient mice is ameliorated after entorhinal cortex lesioning and global ischemia by intraventricular infusion of apoE (88). These animal models may give an indication of the mechanisms that are important in humans.

If early events such as head injury do precipitate AD, a potent acute-phase molecule such as IL1 and the upregulation of neuronal β-APP are necessarily involved with the appearance of diffuse plaques of Aβ-PP widely distributed throughout the cerebral cortex in genetically susceptible (*APOE* ϵ4-positive) individuals (89). These molecular and cellular events are mirrored in the neurodegeneration of AD.

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