Studies of the Choroid Plexus and Its Associated Epiplexus Cells in the Lateral Ventricles of Rats Following an Exposure to a Single Non-Penetrative Blast*

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Summary. The choroid plexus in rats exhibited ultrastructural changes following a non-penetrative blast. The immunophenotypic features of epiplexus cells associated with the choroid plexus epithelium were also altered. In rats killed at 1 and 7 days after the blast, the intercellular spaces between the epithelial cells were greatly widened, coupled with the massive eruption and possible extrusion of the apical cytoplasm into the ventricular lumen. Associated with these changes was the passage of some monocytes/lymphocytes across the epithelium. The incidence of such a migratory phenomenon was more frequent in rats killed 7 days after the blast. In rats killed 14 days after the blast, the ultrastructural changes of the epithelial cells became less pronounced. At 21 and 28 days after the blast, the ultrastructure of the choroid plexus was comparable to that of normal specimens. The immunoreactivity of epiplexus cells in terms of their cell number and staining intensity with the monoclonal antibodies OX-42, OX-18, OX-6 and ED1 was noticeably augmented at 7 and 14 days after the blast; this, however subsided at 21 and 28 days. It is concluded that the choroid plexus is extremely sensitive to a blast wave as manifested by its structural alterations and the vigorous expression of CR3 receptors and MHC antigens by the epiplexus cells. It is suggested that a possible immune response might have been triggered in the cerebrospinal fluid ventricular system following the blast.

Damage to the choroid plexus in the cerebral ventricles has been reported following various types of head injuries. In this connection, the choroid plexus of the third ventricle in humans appears to be damaged more frequently in frontal blows, while the plexus in the lateral ventricles is at a greater risk to lateral blows (GRCEVIC, 1982, 1983 cited by MAXWELL et al., 1992). Structural changes in the choroid plexus, including an increase in number of epiplexus cells (STURROCK, 1978; LING, 1981, 1983), have also been reported in non-human primates after an experimental head acceleration injury (MAXWELL et al., 1992).

The present study was undertaken to assess the structural damage, if any, to the choroid plexus and the cerebrospinal fluid (CSF) ventricular system following a single non-penetrative blast elicited by traditional explosives used in military exercises. Such information may prove useful to better understand the effects of a blast wave on the functioning of the brain as well as providing an anatomical basis for the so-called post-traumatic stress disorder in soldiers as a result of their exposure to different kinds of explosives or gun firings in combat and military training, as studied by many psychiatrists and psychologists (KOLB, 1984; KLEIGER, 1984; FOY et al., 1984). It has been reported that the detonation of explosives in the air produces a region of instantaneous rise in pressure called a blast wave (BROWN et al., 1993) or a shock wave (PODE et al., 1989), which travels radially from the explosive device at a velocity initially greater than the velocity of sound in air (BROWN et al., 1993). The shock wave impacts on and traverses the body without producing external trauma (PODE et al., 1989), but is known to cause injuries to the lungs and tympanic membranes (PODE et al., 1989; BROWN et al., 1993; KROKEenberg et al., 1993). As far as can be

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Fig. 1. Diagram showing the floor plan of the concrete bunker (B) in which the experimental rats were kept in laboratory cages. The explosive was detonated on the ground surface 12.5 m from the floor level of the bunker in which the rat cages (R) were kept. The entrance to the bunker is denoted by E and the stairwell as S.

Fig. 2. Epithelial cells of the choroid plexus in a normal rat. The centrally located nucleus (N) shows a margination of chromatin materials. The mitochondria and cisternae of rough endoplasmic reticulum are widely distributed. Golgi apparatuses (G) are confined to the supranuclear zone. The apical microvilli are slender and uniform in diameter. The intercellular space (IS) between the adjacent cells is moderately widened along the sides of the cells but is obliterated near the apical region where a tight junction (arrows) can be seen. BL basal lamina. Scale bar: 1 μm
Response of Choroid Plexus to a Blast Wave

Ascertained, there has been no available information on the histopathological changes of the choroid plexus or the CSF ventricular drainage system following exposure to a blast. Our recent study (Kaur et al., 1995) reported a widespread activation of microglia as well as atrophic changes in the dendrites of cortical and cerebellar neurons in rats following exposure to a blast. The present study reports the drastic ultrastructural changes of the choroid plexus epithelium, the production site of CSF, in rats subjected to a single blast wave. The epiplexus cells associated with the choroid plexus epithelium (Hosoya and Fujita, 1973; Sturrock, 1978; Ling, 1979), which have been shown to be active macrophages (Lu et al., 1993), also responded vigorously to the blast by upregulating their surface antigens related to endocytosis and immune responses.

MATERIALS AND METHODS

Twenty male Wistar rats weighing about 250 g were subjected to a single non-penetrative blast. The rats were kept in two separate laboratory rat cages (North Kent, England) which were secured to a wooden plank that was further secured to the floor in an underground concrete chamber (dimensions 5 x 6 x 12.5 m, the last being the height of the chamber) simulating a bunker shelter (Fig. 1). The explosive (nitrate based conventional explosive, TNT com-

![Fig. 3. Choroid plexus epithelium 7 days after the blast, showing the widening of intercellular spaces (IS). Arrows indicate tight junctions between the epithelial cells near the apical region. A large pedunculated cytoplasmic protrusion (double asterisks) appears to be extruded into the lumen. The lumen of the ventricle shows a massive accumulation of cytoplasmic fragments (asterisks) containing widely dispersed granular particles. Note also the dilation of the projecting microvilli (MV). Arrowheads indicate the basal lamina. Scale bar: 2 μm]
pound B; 110 kg TNT equivalent) was then detonated on the ground surface, i.e. 12.5 m above the underground chamber in which the animals were kept. This combination of explosive size and distance between explosive charge and animals was selected so that the blast wave effects would be severe but sublethal. The exact parameters were selected based on other considerations, including the strength and stability of the infrastructure as had been tested previously (unpublished data). The rats were sacrificed at various time intervals ranging from 1 day to 28 days after the blast. For controls, 12 normal rats of equivalent body weight were used.

**Electron microscopy**

At 1, 7, 14, 21 and 28 days after experiencing the blast, a pair of surviving rats were perfused with 500 ml of a mixed aldehyde solution composed of 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer following deep anaesthesia achieved with an intraperitoneal injection of 1.5 ml of 7% chloral hydrate. The brains were removed and further fixed in the same fixative overnight at 4°C. One hundred µm thick coronal sections of the brains—which included the two lateral ventricles and choroid plexuses—were cut with a vibratome. Tissue blocks of the choroid plexuses were trimmed and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, for 1 h. Following dehydration in a graded series of alcohol, the tissue blocks were embedded in an Araldite mixture. Ultrathin sections stained in lead citrate were viewed in a Philips CM 120 electron microscope. For comparison, two normal rats were perfused and processed in a similar manner.

**Immunohistochemistry**

For immunohistochemistry, the rats were sacrificed at 1, 7, 14, 21 and 28 days after the blast (n=2 at each of the time intervals). They were deeply anaesthetized with 7% chloral hydrate and then sacrificed by perfusion. The perfusion was preceded with 100 ml of Ringer’s solution until the liver and lungs were clear of blood; this was followed by 500 ml of an aldehyde fixative composed of a mixture of periodate-lysine-paraformaldehyde according to the method by MCLEAN and NAKANE (1974), but with a concentration of 2% paraformaldehyde. The perfusion lasted for 15 min, after which the brains were removed and kept in

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*Fig. 4.* Choroid plexus epithelium 7 days after the blast showing a sessile cytoplasmic protrusion (*asterisk*). Scale bar: 1 µm

*Fig. 5.* Cytoplasmic fragments in the ventricular lumen 7 days after the blast. One of the fragments (*asterisk*) shows tubular profiles resembling cisternae of the endoplasmic reticulum (*arrows*). Scale bar: 1 µm
a similar fixative for 2 h. The brains were then kept in 0.1 M phosphate buffer containing 10% sucrose overnight at 4°C. Sets of four of frozen coronal sections of the brains—including the two entire lateral ventricles and their choroid plexuses—were cut serially at 40 μm thickness and rinsed in phosphate-buffered saline (PBS). Consecutive sections were mounted on different slides and then incubated at room temperature respectively with the monoclonal antibodies OX-42 (Sera-Lab MAS 370b), OX-18 (Sera-Lab MAS 101B) and OX-6 (Sera-Lab MAS 143b) diluted 1:100, and ED1 (Serotec MCA 341) diluted 1:400 with PBS for 18 h according to the protocol described previously by us (Lu et al., 1994). These antibodies served for the detection of complement type 3 receptors (CR3), major histocompatibility complex class I (MHC I) and II (MHC II) antigens and cells of monocyte/macrophage lineage, respectively. A vectastain ABC-kit (PK-4002, Vector Laboratories, USA) against mouse IgG was used for subsequent detection of antibodies with 3,3’-diaminobenzidine as a substrate. For control incubations, some sections were incubated in a medium by omitting the primary antibodies; in all these, the immunoreaction was abolished. For comparison, normal rats of equivalent body weight were sacrificed in pairs at each time interval and processed for immunohistochemistry as above.

For quantitative study, the sections immunostained for OX-42 from all rats were used. A total area of 0.25 mm² from ten sections of the choroid plexus as
outlined with an ocular grid measuring 0.0125 mm² was surveyed in each rat. The choroid plexuses of both lateral ventricles were scrutinized. All OX-42 positive epiplexus cells associated with the choroid plexus epithelium were enumerated directly under the photomicroscope at a magnification of 400 times.

**RESULTS**

**Ultrastructure of the choroid plexus**

**Normal rats**

The choroidal epithelial cells in normal (control) rats formed a simple cuboidal epithelium resting on a layer of basal lamina. Each cell contained a pale nucleus either centrally located or placed close to the base (Fig. 2). The cytoplasm was endowed with widely distributed mitochondria, rough endoplasmic reticulum, several Golgi apparatuses and some vesicles near the supranuclear region (Fig. 2). Large numbers of tightly packed microvilli of uniform diameter projected into the ventricular lumen (Fig. 2). Intercellular spaces between the lateral walls of the epithelial cells were evident (Fig. 2). At the apical region the contiguous epithelial cells formed tight junctions wherein the intercellular space was obliterated (Fig. 2).

**Experimental rats**

All rats appeared physically normal and active after the blast. Examination of the walls of the lateral

Figs. 9-17. Epiplexus cells (arrows) numerous and intensely stained for OX-42 (Figs. 10, 11), OX-6 (Fig. 13), OX-18 (Fig. 15) and ED1 (Fig. 17) 7 days after the blast when compared with their corresponding controls in normal rats stained with OX-42 (Fig. 9), OX-6 (Fig. 12), OX-18 (Fig. 14) and ED1 (Fig. 16). A majority of the epiplexus cells are ramified with extended processes as shown by the tangential section in Fig. 11. Scale bar: 50 μm in Figs. 9, 10, 12-17; 30 μm in Fig. 11.

Fig. 18. Lateral ventricle of a rat 1 day after the blast showing two OX-6 positive cells (arrows) passing through the ependymal lining (E). Scale bar: 30 μm
Figs. 9–18. Legends on the opposite page.
ventricles showed that the lining ependyma remained structurally intact. Histological changes at the choroid plexus, however, were evident as early as 1 day after the blast. The most striking alteration was the considerable widening of the intercellular spaces between the adjacent choroidal epithelial cells resting on a basal lamina which appeared normal (Fig. 3). The microvilli of some of the cells were dilated at their free ends. Another remarkable feature was the massive eruption of cytoplasmic protrusions at the apical surface of the cells; these appeared either pedunculated or sessile (Figs. 3, 4). Many of the cytoplasmic protrusions appeared to be extruded or detached from the cell surface (Figs. 3, 5), so that in some areas the lumen of the ventricle was filled with fragments of cytoplasm. Some of the cytoplasmic fragments contained tubular profiles resembling cisternae of endoplasmic reticulum and granular particles (Fig. 5). Isolated profiles of cytoplasmic fragmentation were often observed in the vicinity of epi-plexus cells containing phagosomes (Fig. 6).

At 1 and 7 days after the blast, a variable number of monocytes/lymphocytes were observed to be lodged in the intercellular spaces (Fig. 7) of the epithelial cells. They were characterized by a nucleus with dense chromatin clumps (Fig. 7); their small amount of cytoplasm showed some dense granules, mitochondria and cisternae of rough endoplasmic reticulum. Very often, the cells were separated from the ventricular lumen by only a tenuous layer of epithelial cytoplasm.

At 14 days after the blast, the intercellular spaces between adjacent epithelial cells became less dilated. Except for some cells whose cytoplasm was extremely vacuolated and showed dilated microvilli (Fig. 8), most of the cells displayed normal features. Inter-epithelial mononuclear cells were not observed at this stage.

In longer surviving intervals, i.e., in rats killed at 21 and 28 days after the blast, the choroid plexus displayed features comparable to those of the normal rats. Apical cytoplasmic protrusions were no longer evident and the projecting microvilli appeared normal.

**Immunohistochemistry**

The present immunohistochemical study focused on the epi-plexus cells residing on the ventricular surface of the choroid plexus epithelium because they were selectively marked by the various monoclonal antibodies used. One day after the blast, these cells did not show any noticeable difference from those of the control rats in terms of their numbers and immunoreactivity with OX-42, OX-18, OX-6 and ED1.

At 7 and 14 days after the blast, the epi-plexus cells which appeared to have increased in numbers were strongly immunostained with OX-42, OX-18, OX-6 and ED1 when compared with the corresponding control or normal animals (Figs. 9–17). In the latter, the immunoreactivity of the epi-plexus cells was less intense and, furthermore, the cells were more sparsely distributed (Figs. 9, 12, 14, 16). A majority of the immunoreactive cells in control and experimental rats were ramified notably on their surface view (Fig. 11). A noteworthy feature in the experimental rats at this stage was the common occurrence of OX-42 and OX-6 positive cells in the process of migration across the ependymal lining (Fig. 18).

The elevated immunoreactivity of epi-plexus cells with OX-42, OX-18, OX-6 and ED1 was sustained in rats killed at 21 days when compared with that of the normal control rats. It was, however, less intense when compared with the experimental rats killed at 7 or 14 days after the blast. At 28 days after the blast the immunoreactivity as well as the frequency of the epi-plexus cells was comparable to that of the control rats.

The quantitative study showed that the number of OX-42 positive epi-plexus cells at 7 and 14 days after the blast was considerably increased when compared to the controls, whereas in 1, 21 and 28 days after the blast, the number of cells was comparable to the controls (Table 1).

<table>
<thead>
<tr>
<th>Rat</th>
<th>OX-42 positive cells (mean ± SD)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>145.5 ± 6.36</td>
</tr>
<tr>
<td>1 day post blast**</td>
<td>157.0 ± 2.82</td>
</tr>
<tr>
<td>7 days post blast*</td>
<td>240.5 ± 3.55</td>
</tr>
<tr>
<td>14 days post blast*</td>
<td>247.5 ± 3.53</td>
</tr>
<tr>
<td>21 days post blast**</td>
<td>177.0 ± 8.48</td>
</tr>
<tr>
<td>28 days post blast**</td>
<td>155.5 ± 7.77</td>
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</tbody>
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*indicates that the number of OX-42 positive cells is significantly higher than in the normal rats as analysed by the Student's T-test (two-tailed) on the EPISSTAT programme; **indicates that the value is not significantly different from that of normal rats.
DISCUSSION

It is unequivocal from this study that the choroid plexus and its associated epiplexus cells underwent drastic structural alterations when exposed to a single non-penetrative blast. The rats did not show any signs of external trauma, suggesting that despite its being deeply embedded in the CSF, the choroid plexus is extremely vulnerable to a blast force. The earliest sign of lesion at the choroid plexus was manifested in rats killed at 1 day and intensified at 7 days after the blast, when the intercellular spaces between the epithelial cells were considerably widened. Such a feature is reminiscent of what has been described in postnatal rats given intraperitoneal or intravenous injections of horseradish peroxidase (LU et al., 1993), in which the lateral cell walls of the choroid plexus epithelium became extremely convoluted for the active transport of exogenous material across the epithelium. It is therefore suggested that the widened intercellular spaces here described may also be involved in the enhanced transepithelial transport of materials and secretion into the cerebrospinal fluid following the blast. Associated with this change was the common occurrence of mononuclear cells, monocytes/lymphocytes, lodged in the intercellular spaces of the epithelial cells, suggesting their transepithelial migration. Such a phenomenon was reported in baboons after experimental head acceleration injury (MAXWELL et al., 1992). Interestingly, the transepithelial migration of mononuclear cells had never been observed in normal rodents (LING, 1979) except when they were challenged with the bacterial toxin, lipopolysaccharide (LPS) (LU et al., 1994). The occurrence of the transepithelial passage of monocytes coupled with the increase in the number of ED1 immunoreactive cells in the present study after the blast suggests a rapid influx of these cells probably taking place in response to a greater demand of epiplexus cells in the CSF ventricular system. The occurrence of OX-42 and OX-6 positive cells penetrating the ependymal lining of the ventricular walls after the blast would be an additional route of entry of immunoneactive cells from the parenchymal microglial cells contributing to the increase of intra-ventricular macrophages. It is conceivable that the infiltrated cells would first take the supraependymal position as the supraependymal cells and subsequently migrate across to become the epiplexus cells. An upsurge in epiplexus cells had previously been reported in head injury, e.g., after experimental hydrocephalus (NIELSEN and GAUGER, 1974), or after penetrant cerebral lesion (MAXWELL and McGAHEY, 1988). Since epiplexus cells are known to be phagocytes endowed with immuno-molecules characteristic of antigen presenting cells (LING, 1985; LU et al., 1994), their increase in number following the blast suggests their involvement in an active phagocytic function and immunological response that may have been elicited by the blast.

The epithelial cells of the choroid plexus displayed an unusual feature after the blast in which their apical surface was thrown into numerous cytoplasmic protrusions. It is possible that some of them were subsequently extruded into the ventricular lumen as many cytoplasmic fragments bearing similar features were observed far removed from the apical cell surface. Only rarely were the detached cytoplasmic fragments engulfed by the epiplexus cells, if they were indeed extruded from the epithelial cells. The extrusion of cytoplasmic materials into the cerebrospinal fluid by the choroid plexus epithelial cells was also observed in cats following a cisternal injection of a crotoxin complex (Phospholipase A2) (LING et al., 1988). The significance of a possible enhanced CSF secretion by the choroid epithelial cells following the blast remains uncertain, although from a speculative point of view it is possible that the metabolic activity of the choroid plexus would have increased.

In conclusion, the present results showed that a single non-penetrative blast elicits a transient structural alteration of the choroid plexus epithelium coupled with a vigorous upregulation of CR3 receptors, MHC class I and II antigens and ED1 antigens on epiplexus cells. All these features may be related to increased receptor mediated endocytosis and a possible immune response of epiplexus cells after the blast. This is further evidenced by the entry of monocytes/lymphocytes in our ultrastructural observations. The epiplexus cells also responded by increasing their cell population as asessed by our crude cell counts. It remains uncertain whether these changes are due to a direct effect of the blast or secondary to some biochemical alterations in the CSF. In any event, the effect of the blast wave is reversible in nature as evidenced by the recovery of the choroid plexus to its normal appearance in longer surviving rats.

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