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Lipopolysaccharide Aggravates Cerebral Pathology in B10.PL-derived CD1-/-, β_2m-/-, TCRα-/-, and TCRδ-/- Knockout Mice*

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Adult B10.PL-derived immunological genes knockout mice injected with 100 μg lipopolysaccharide (LPS) showed severe hydrocephalus and meningitis. A consequence of the hydrocephalus is pineal hyperplasia, sponginess of periventricular parenchyma, gliosis and, at the last stage of hydrocephalus formation, disappearance of the ependymal layer and the Gomori-positive subependymal astrocytes. Possible mechanisms for the aggravation of cerebral pathology induced by LPS are discussed.

Key words: LPS, hydrocephalus, meningitis, pineal tumors, knockout mice.

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Septic shock, the most severe problem of sepsis, is a lethal condition caused by the interaction of a pathogen-induced long chain of sequential intracellular events in immune cells, epithelium, endothelium, and the neuroendocrine system. The lethal effects of septic shock are associated with the production and release of numerous pro-inflammatory biochemical mediators including cytokines, nitric oxide and toxic oxygen and nitrogen radicals, together with the development of massive apoptosis (CARILLO-VICO *et al.* 2005). CHING *et al*. (2006) showed that systemic inflammation induced by LPS treatment actively inhibits recruitment of leukocytes by CNS. Previously, it was found that pathogen-free CD1-/-, β_2m-/-, TCRα-/-, and TCRδ-/- B10.PL-derived knockout mice** have cerebral pathology in the form of intravascular coagulation, microhemorrhages, serous exudates in the cerebral ventricles and in the subarachnoid space, and prominent hydrocephalus, which is often accompanied by pineal hypertrophy (SURA & SREBRO 2005). In the present study, a comparison is made of morphological changes in brains of mice with a selective lack of T cell populations that were treated intraperitonealy with LPS. LPS is the active immunostimulant in the cell wall of Gram-negative bacteria responsible for triggering the cascade of events following bacterial infection, resulting in secretion of a variety of potent mediators and cytokines produced primarily by activated macrophages and monocytes (KIELIAN & BLECHA 1995). LPS also induces the tissue factor (TF), which is the major *in vivo* activator of blood coagulation leading to thrombin generation and fibrin deposition (ERLICH *et al.* 1999). The astrocytes are the prime source of TF, at least in the murine central nervous system (EDDLESTON *et al.* 1993). The brain must use efficient mechanisms to limit hemorrhage. Intracranial bleeding is often toxic to neuronal function, altering the neural microenvironment formed by the blood-brain barrier; thus the hemorrhage resulting from cerebrovascular disease or acute brain trauma can result in paralysis, coma, and

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**TCRα-/- mice – mice that lack TCR$\alpha\beta$ T cells, TCRδ mice – mice that lack TCR$\gamma\delta$ T cells, β_2m-/- mice – mice that lack CD8 T cells and CD1-/- mice – mice that lack CD1-restricted NKT cells.

death (EDDLESTON *et al*. 1993). In untreated mice, TF mRNA was also detected in placenta, lung, renal glomeruli, and cardiomyocytes in the heart (ERLICH *et al*. 1999; MACKMAN *et al*. 1993). LPS affects the passage of other proteins across the blood-brain barrier (BBB) through its release of cytokines and disruption of the BBB (NONAKA *et al*., 2005). Hydrocephalus is a consequence of intracerebral fluid accumulation due to disrupted BBB (YAN *et al*. 2004; THOMÁS-CAMARDIEL *et al.* 2004).

The results show that LPS administration considerably aggravates the cerebral pathology observed in B10.PL mice and their immunologic gene KO derivatives.

Material and Methods

Animals. Six to eight week old SPF female B10.PL (H-2^u) mice were obtained from the Jackson Laboratory, Bar Harbor, ME. In some experiments the following immunodeficient mice: TCRα-/-, TCRδ-/-,CD1-/- and β_2m-/- on H-2^u background were used. B10.PL and all knock out mice were received as a gift from Dr. C. A. JANEWAY at Yale University School of Medicine. Then mice were bred in the animal facility at the Jagiellonian University, College of Medicine. All mice were fed autoclaved food and water, kept under pathogen-free conditions using filter-topped microisolator cages and sterile equipment. Experiments were carried out according to guidelines of the Animal Use and Care Committee of Jagiellonian University.

Reagents. Lipopolysaccharide (LPS) from *E. coli* 026:B6 was obtained from Sigma Chemical Co., St Louis MI.

Treatment with LPS. Wild type B10.PL and TCRα-/-, TCRδ-/-,CD1-/- and β_2m-/- knockout mice on H-2^u background were intraperitonealy injected with one dose of 100 μg of LPS in 1 ml of sterile PBS. In control groups animals were i.p. injected with PBS alone. Each experimental and control group contained 14 mice. Animals from the same strains not subjected to LPS treatment served as controls. The animals were killed at 24h to 28 days post treatment.

Brain isolation and histological assessment. Their brains were quickly exposed by removal of the skin and skull vault and fixed *in situ* with Bouin's fluid for 24h. Serial paraffin-embedded sections of the pros- and mesencephali were stained with Gomori's chrome hematoxylin-phloxin (PEARSE 1960) or the Giemsa stain.

Results

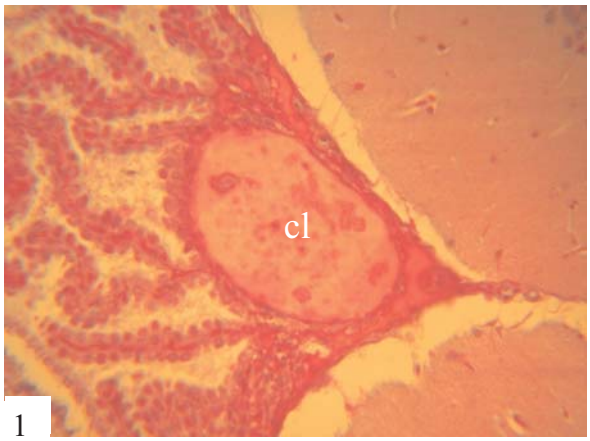
Intravascular serous clots were regularly observed in all experimental (LPS-treated) (Figs 1 & 2) and, to a lesser degree, in the untreated control mice. The clots contained macrophages, and less frequently, granulocytes. Serous exudates and clots were observed in the ventricles and the cerebral aqueduct (Figs 3, 4, & 5). Microhemorrhages occurred mainly in the subarachnoid space and, sometimes, in the ventricles (Fig. 6). The hemorrhagic foci contained hemosiderin deposits in the form of small, medium size, and large granules (Figs 7 & 8). Hemosiderin deposits were present in the lumina of meningeal arteries and meningeal and parenchymal veins of medium and large size. The latter were dilated with signs of blood stasis.

Medium grade hydrocephalus (Fig. 9) was observed in all experimental groups and, with low or medium grade severity, in the untreated controls. The severity of the hydrencephali increased at later post injection intervals, the brain at this stage showing subventricular spoginess (Fig. 10) with disappearance of the ependyma, loss of neurons and gliosis. Qualitatively the pathologies observed in the different experimental groups were similar. However, quantitative differences were evidently present: The TCRα-/- animals had the most severe hydrocephalus, less severe being observed in the CD1-/-, β_2m-/-, and TCRδ-/- individuals.

Thickening of the meninges with mast cell infiltration was a common finding after LPS treatment. However, parenchymal mast cells in the thalamus, sporadically observed at earlier stages p.i., later completely disappeared.

Astrocytes, one of the type of the Gomori-positive glia, are localized periventricularly and

Figs 1-8. Fig. 1. Intravascular clot (cl) in large vein at the base of the choroid plexus of the third ventricle of LPS-treated CD1-/- mouse after 24 days postinjection. Chrome hematoxylin-phloxin (CHP), × 400. Fig. 2. Intravascular clot (cl) in large vein at the base of the choroid plexus of the third ventricle of LPS-treated B10.PL mouse 5 days postinjection. CHP, × 400. Fig. 3. Intraventricular serous clot (cl) in the upper part of the third ventricle of LPS-treated CD1-/- mouse 5 days postinjection. CHP, × 200. Fig. 4. Serous clot (cl) in the lateral ventricle of LPS-treated β_2m-/- mouse 10 days postinjection. CHP, × 200. Fig. 5. Serous clot (cl) in the lateral ventricle of a non-treatet β_2m-/- mouse. CHP, × 400. Fig. 6. Intraventricular microhemorrhage (mh) in LPS-treated β_2m-/- mouse 10 days postinjection. CHP, × 400. Fig. 7. Hemosiderin (hs) deposits in the upper part of the third ventricle in LPS-treated β_2m-/- mouse 10 days postinjection. Giemsa, × 200. Fig. 8. Hemosiderin (hs) deposits in the upper part of the third ventricle in LPS-treated CD1-/- mouse 15 days postinjection. Giemsa, × 400



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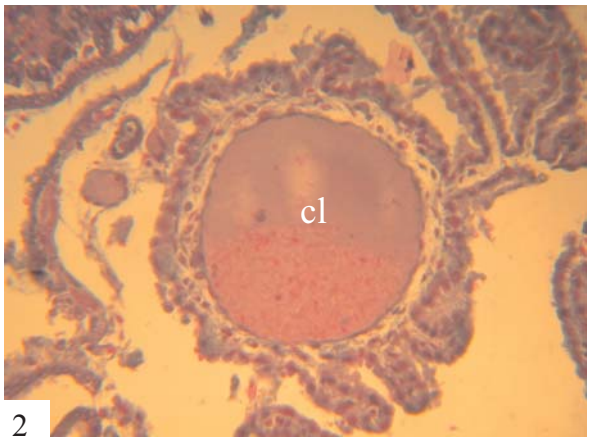

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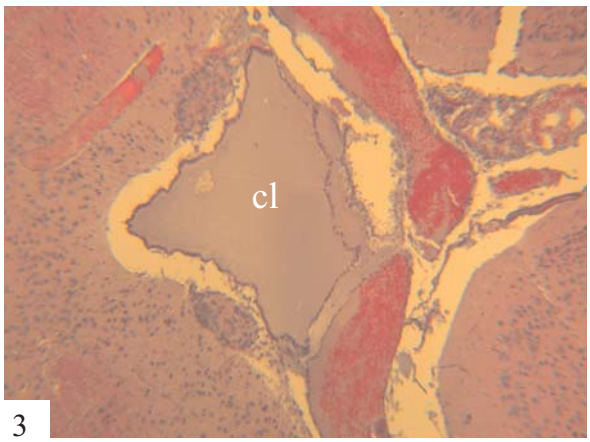

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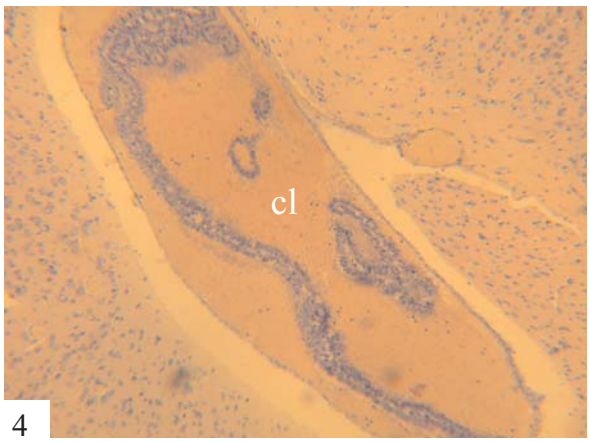

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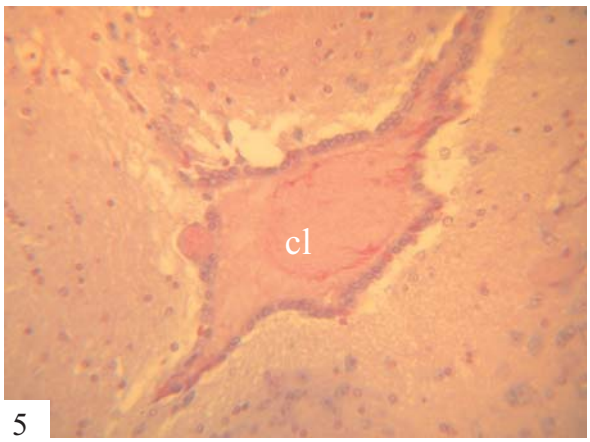

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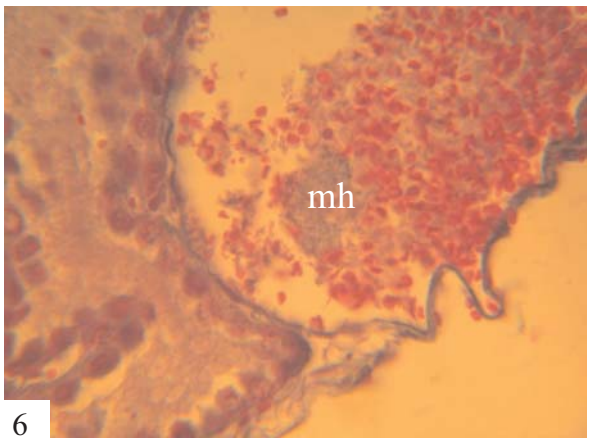

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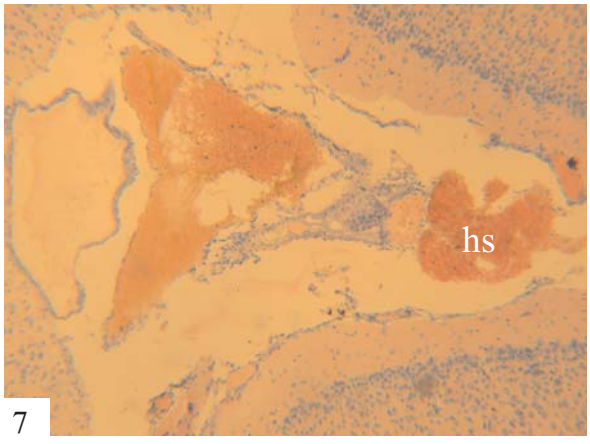

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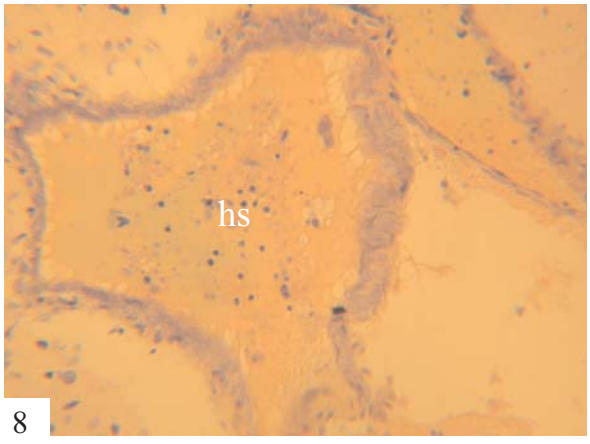

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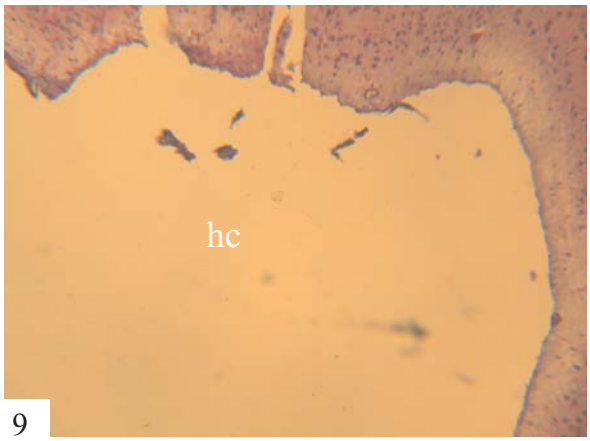

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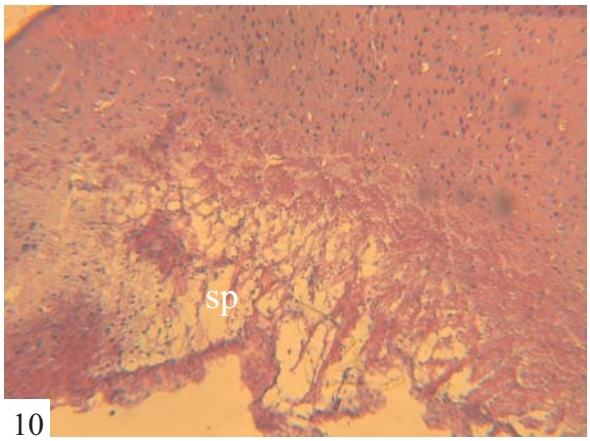

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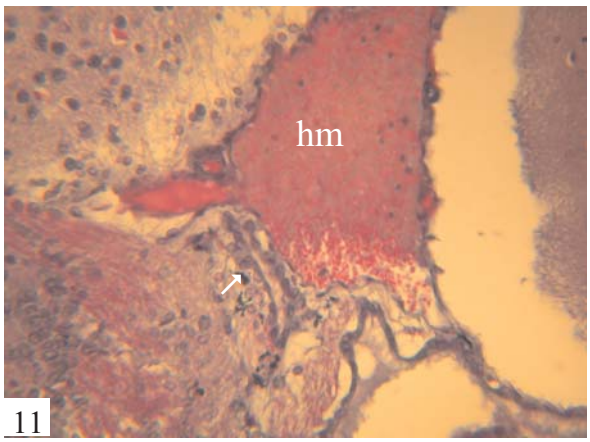

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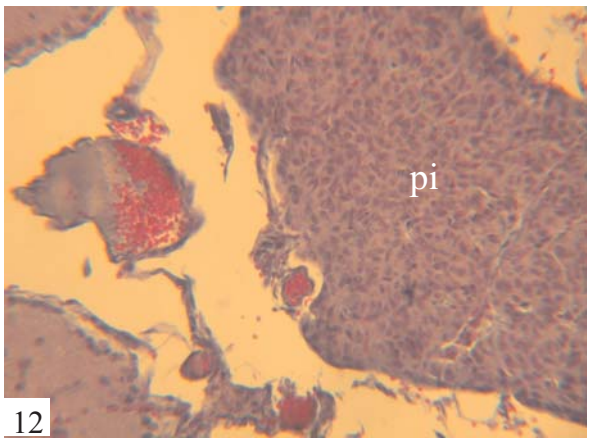

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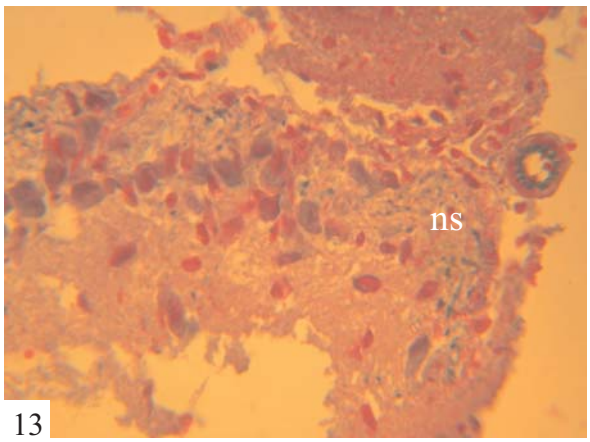

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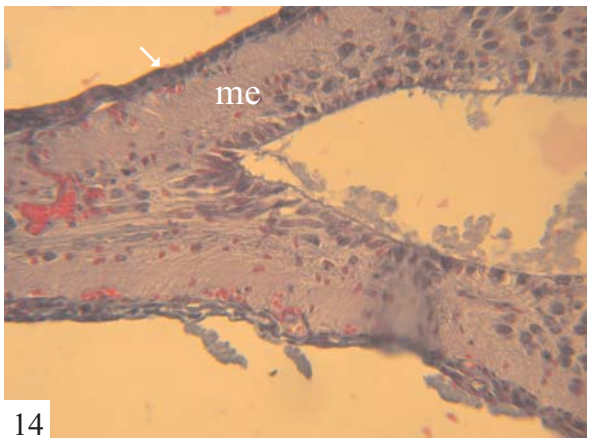

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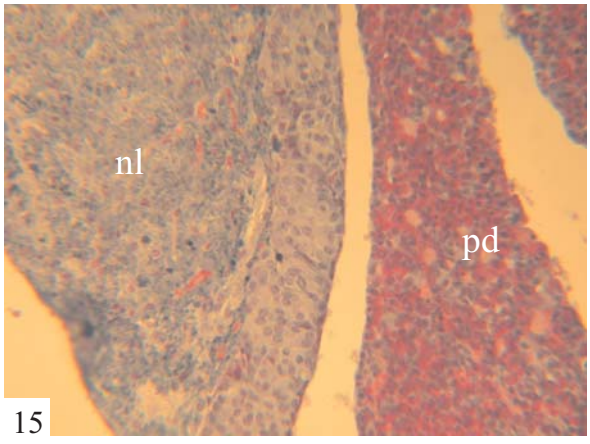

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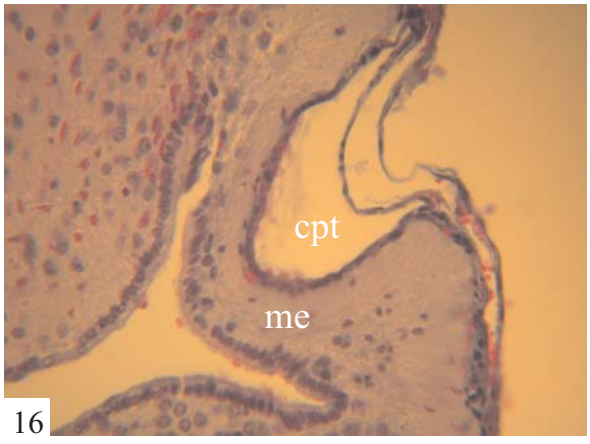

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subependymally. They contain a cytoplasmic material staining with Gomori's chrome hematoxylin phloxin and rich in reducing sulfur (SREBRO & SURA 2002). In our experimental animals these cells were more numerous and the sulfur-rich material was more abundant in the LPS-treated mice, particularly at sites near the intravascular clots and intraventricular hemorrhages (Fig. 11).

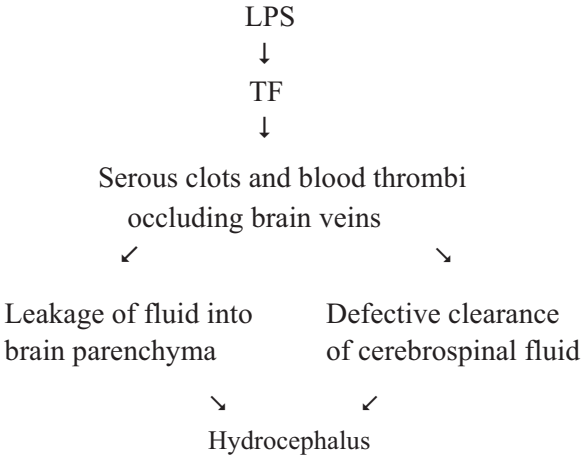
Pineal gland hyperplasia and hypertrophy (Fig. 12) in the form of a benign tumor was observed in approximately 50% of the experimental animals, the controls showing a frequency of ca. 10%. These tumors constricted the central brain vein (Galen's vein).

The vasopressin-producing neurosecretory system of the hypothalamus was hypoactive in all animals showing severe hydrocephalus. This hypoactivity was judged from the small size of the neurosecretory cells of the supraoptic and paraventricular nuclei and piling up of the neurosecretory material in the neurosecretory fibers of the hypothalamus (Fig. 13), the median eminence (Fig. 14), and the neural lobe (Fig. 15). The vessels of the portal system showed blood stasis and the pars tuberalis of the pituitary gland was involuted with small flat cells and huge cysts (Fig. 16).

Discussion

The present results show that the B10.PL mice are subject to spontaneous intravascular coagulation and, consequently, develop a hydrocephalus. This tendency is even greater in the B10.PL-derived immunological genes knockout mice (SURA & SREBRO 2005). Administration of LPS to such mice aggravates this brain pathology where severe hydrocephalus is formed concomitant and, probably, as consequence of vast intravascular coagulation, serous intraventricular and subarachnoid exudates, and microhemorrhages. The intravascular coagulation most probably is a consequence of TF formation in response to the LPS (EDGINGTON *et al*. 1992; CHU *et al.* 2002; LWALEED *et al*. 2001). LPS also causes a breakdown of the BBB (TOMÁS-CAMARDIEL *et al.* 2004) and induces infiltration of mast cells in the *dura mater*. Histamine released by mast cells causes microvascular leakage in pial venules (TORE *et al.* 2001), where mast cells regulate blood flow and vessel permeability (YONG *et al*. 1994). Low doses of LPS induce the entrance of blood plasma albumin into brain parenchyma (PORZIONATO *et al*. 2004; YAN *et al*. 2004).

On the basis of literature data and our former and present results the following sequence of events leading to the formation of the hydrocephalus is suggested:



A consequence of the hydrocephalus is pineal hyperplasia due to a lack of negative feedback from the defectively circulating cerebrospinal fluid, sponginess of periventricular parenchyma with loss of neurons, gliosis and, at the last stage of hydrocephalus formation, disappearance of the ependymal layer and the Gomori-positive subependymal astrocytes.

Acknowledgements

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Figs 9-16. Fig 9. Distended lateral ventricle (hc) in hydrocephalic brain of a non-treated β_2m-/- mouse. CHP, × 200. Fig. 10. Vast subependymal sponginess (sp) of the brain parenchyma in a hydrencephalic brain of the LPS-treated TCRδ-/- mouse 21 days postinjection. CHP, × 200. Fig. 11. Astroglial Gomori-positive masses (arrow) near an intraventricular hemorrhage (hm) in LPS-treated CD1-/- mouse 15 days postinjection. CHP, × 400. Fig. 12. Pineal tumor (pi) in LPS-treated β_2m-/- mouse 15 days postinjection. CHP, × 200. Fig. 13. Accumulation of Gomori-positive (vasopressin-carrying) neurosecretory material in the supraoptic nucleus (ns) of the hydrencephalic control BALB/c mouse. CHP, × 400. Fig. 14. Median eminence (me) in an hydroencephalic LPS-treated B10.PL mouse 5 days postinjection. The median eminence is distorted and the pars tuberalis (arrow) involuted. CHP, × 200. Fig. 15. The hypophysis in an hydrencephalic LPS-treated β_2m-/- mouse 24 days postinjection. The neural lobe (nl) is full with the Gomori-positive neurosecretory material, the pars distalis (pd) showing extensive eosinophilia. CHP, × 200. Fig. 16. A distorted median eminence (me) and a huge cyst in an involuted pars tuberalis (cpt) in LPS-treated β_2m-/- mouse 24 days postinjection. CHP, × 400.

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