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[A1]

Mast cells in human brain pathology

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Mast cells (MC) are derived from haematopoietic progenitor cells that enter nearly all vascularized tissues, where they complete their maturation and, under some circumstances, can produce a spectrum of proinflammatory mediators, including certain cytokines and chemokines. Antigens that initiate the activation of mast cells have three outcomes: degranulation, with the secretion of preformed mediators that are stored in the cytoplasmic granules of the cell; the *de novo* synthesis of proinflammatory lipid mediators; and the synthesis and secretion of cytokines and chemokines.

Mast cells can be activated to express effector functions by many mechanisms, and these cells are now thought to participate in a wide variety of physiological and pathological processes.

Mast cells have been shown to be essential for certain innate immune responses, and they have been implicated as potentially crucial effectors in many other settings such as angiogenesis, host responses to neoplasia and certain autoimmune disorders.

Recent studies have shown the importance of these cells (compared with other potential effector cells) for chronic inflammation and other long-term tissue changes.

In contrast to some animal species, in normal human brains of foetuses and adults MC are few in number and it is not clear in which brain pathological responses these cells participate in humans.

In the present study we show the early events concerning the infiltration of mast cells into the brains of intoxicated patients and the accumulation of these cells in brains with tumours and following infections.

The study was performed on human brains obtained following autopsy. They include brains of patients with infections, intoxications, neoplasia and brain congenital malformations. Paraffin blocks of brain specimens were drawn from the files (1970–2003) of the Department of Developmental Neuropathology of the Medical Research Centre in Warsaw. Serial sections were examined for MC phenotypes and for the capillary network by immunohistochemistry. Primary mouse monoclonal antibodies to tryptase and chymase (Chemicon, USA; diluted 1:100) and biotinylated Ulex Europeus lectin (Vector Lab,USA; diluted 1:500) were applied to the tissue sections. Immunoreactions were visualised using biotinylated secondary antibodies: goat anti-mouse or horse anti-mouse and an alkaline phosphatase-avidin-biotin conjugate or ABComplex/HRP (DAKO, UK). Dual localisation of chymase and trypatase was also studied.

Number of mast cells was counted in each tissue section under the light microscope Nikon microphot--FXA with magnification x 100 in 10–50 fields depending on the size of the brain section. The data from three serial sections of each paraffin block were pooled and mean values were calculated.

Negative controls: primary antibodies were replaced with an appropriate isotopic normal mouse immunoglobulin fraction at matched protein concentration. These were included for the examination of each specimen and consistently produced negative results.

For ultrastructural localization of tryptase, the incubation of ultra-thin sections with the primary antibody was followed by incubation with the secondary antibody coupled with gold particles.

The results of our present study confirm previous observations that, independently of age, MC residing in the normal human brain are not numerous and are mainly MC_{TC} phenotype.

In all brains affected by infections, intoxications and tumours that were used for this study, an increased number of MC was found. The tryptase mast

cell phenotype (MC_T) predominated (95%) over the tryptase-chymase mast cell (MC_{TC}) phenotype (5%).

The early events concerning the infiltration of tryptase mast cells were observed in the brains of intoxicated patients who died within 3–5 days after exposure to the toxic agent. Mast cells accumulated in the blood and adhered to the endothelium of the brain vessels. Then they infiltrated the brain parenchyma around the vessels or penetrated throughout the brain area affected by the pathology. These mast cells have characteristic ultrastructure and their cytoplasmic granules contain tryptase. In some brain regions such mast cells may easily release their contents forming tryptase-immunopositive large patchy areas.

The tryptase mast cells that are implicated in the brain tumours (sclerosis tuberosa, haemangioblastoma, menigiomas) and stay for a long time in the tissue are usually large and of the regular round-shape cells. Those and other MC_T that infiltrate the immediate surroundings of the tumours (neurocysticercosis) may be activated and then they become cells of irregular shape with long cell processes surrounded by numerous small grains or a diffuse immunoreactive halo, depending presumably on the mechanism of cell stimulation.

The results of our study show that independently of the patient's age, numerous tryptase mast cells infiltrate the brains that are affected by the different pathological processes. In the acute tissue response, these cells may easily release the content of their cytoplasmic granules. Moreover, those tryptase mast cells that are implicated in brain tumours or stay for a long time around the tumours may be suddenly activated and involved in the pathogenesis of the brain disease.

[A2]

Extensive mixed vascular malformation clinically imitating multiple sclerosis – case report

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Vascular malformations usually develop as a result of influence of teratogenic factor(s) acting in the defined embryonic/foetal period. However, in the case examined by us, various types of vascular malformations formed in different periods of ontogenic development were found. They were seen in all parts of the central nervous system and clinically mimicked multiple sclerosis. Against the background of generalized ischaemic lesions of the CNS, certain kinds of vascular malformations were seen: cavernous or foetal-like vessels within meninges, superficially located capillary angioma penetrating into the brain and spinal cord white matter, and arterio-venous pathological conglomerates forming meningeal angiomatosis. Examination of the vascular basal membrane compounds revealed poor immunoreactivity to laminin and fibronectin. There were no disturbances in expression of trophic vascular factors. The presence of various types of pathological vessels originating from different ontogenic periods indicates remittent or prolonged influence of teratogenic factor(s) in all periods of foetal vessel development.

[A3]

Serum leptin levels in rats with brain lesions caused by neonatal hypoxia-ischaemia

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Leptin is a protein originally reported to be a hormone identified as an important regulator of energy metabolism and adipose stores. Leptin also exhibits a variety of other effects, including the regulation of endocrine function, reproduction, immunity and inflammation. In addition, several lines of evidence show that leptin regulates the hypothalamic-pituitary-adrenal (HPA) axis.

The role of leptin in brain development is documented by the presence of neuroendocrine and structural neuronal abnormalities in ob/ob mice with

genetic leptin deficiency. In normal mice, during the early postnatal period, serum leptin levels are higher than in animals after weaning. In this period, the temporal relationship between leptin and other hormones is regulated in a manner distinct from that observed in adult animals. It has been suggested that the immature response of the HPA axis to various stressors may stabilize circumstances of neonatal development. However, in this period of life some animals are susceptible to asphyxia, which may cause serious brain injury. In the present study the effect of neonatal hypoxia-ischaemia (asphyxia) on serum leptin levels in rats with brain lesions was examined.

The study was performed on Wistar rats. The 7-day-old pups were anaesthetized with halotan and cerebral hypoxia-ischaemia (HI) was induced by a previously described technique. At various time intervals after the exposure, HI rats and age--matched control pups were anaesthetized and blood was obtained from their heart. Animals were weighed prior to sampling. Serum was stored at –20°C until assayed. Afterwards, pups received a transcardiac infusion of 4% paraformaldehyde solution, then their brains were removed, immersed in the above-described fixative for 24 h at room temperature, dehydrated in alcohol, and embedded in paraffin. Deparaffinized and hydrated sections were washed in PBS and stained with cresyl violet. Leptin was measured in serum samples by radioimmunoassay using a rat Leptin RIA Kit (Linco Research). All values were expressed as mean ±SEM. The statistical significance of changes of the various parameters measured was assessed at the 5% level using ANOVA and Student-Newman-Keuls method for multiple comparisons where appropriate.

In a group of HI pups with brain lesions, changes were largely restricted to the cerebral hemisphere ipsilateral to the common carotid artery occlusion. They involved the cerebral cortex, subcortical and periventricular white matter, striatum (basal ganglia) and hippocampus. During the first 3 days after HI exposure, brain oedema increased progressively and in subsequent days tissue injury appeared in the form of either neuronal necrosis or infarction seen in the cerebral cortex and basal ganglia (striatum and thalamus) with secondary tissue autolysis. The cerebral cortex was reduced greatly in size or to a thin membrane bordering either a clear cystic space or expanded lateral ventricle. Moreover, our results confirm observations of other authors that leptin level in the second postnatal week of life is higher than that in the weaning animals. However, in HI pups we do not observe such leptin profile and leptin levels during the whole period of recovery after hypoxic-ischaemic exposure are lower than in age-matched control rats. The body weight of these pups progressively increased as in the group of control animals.

Leptin is a protein originally characterized as the "satiety hormone" which is a part of a signalling pathway from adipose tissue to the brain. In adult animals leptin plays a role in control of appetite and body weight. Our present findings confirm the previously published hypothesis that during postnatal period, leptin has developmental and neuroendocrine actions distinct from those related to energy homeostasis.

In conclusion, leptin deficiency found in our HI neonatal rats suggests that during the period of healing, animals with brain lesions may require supplementation with exogenous leptin to normalize the hormonal balance characteristic for neonatal early development.

[A4]

Comparison of neuronal migration disturbances in cerebral and cerebellar hemispheres

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Migration of neuronal progenitors from proliferative zones to their final location in the cerebellar and cerebral cortex is a key event during development. Sometimes abnormal groups of neurons are arrested in the white matter of hemispheres during their way to the cortex. The aim of our study was: 1) to investigate the picture of these arrested groups of neurons in the cerebellar and cerebral hemispheres; 2) to investigate the influence of this failed migration upon cortical layering and gyrus formation. The analyzed material comprised 820 infantile cases whose brains were examined in the Department of Developmental Neuropathology during

1984–2004. Brains with abnormal clusters of neurons in the white matter of the hemispheres were chosen. In the white matter of the cerebellum, heterotopias were found in 34 cases (age 28 gestational weeks – one and a half year), and in 5 cases (age 22 gestational weeks – 5 years) in the cerebral white matter.

Among cerebellar heterotopias 15 were found in the vermis, 12 in the flocculus, 15 in the hemispheres and 11 in the dentate nucleus. In 5 cases abnormal clusters of grey matter were observed in all three localizations. The groups of neurons within the white matter were located periventricularly, centrally, or subcortically.

Several types of cerebellar heterotopias can be distinguished relating to their morphological picture:

1) rounded clusters of grey matter with preserved cortical pattern. The distinct, evident molecular layer with nests of external granular layer cells was surrounded by the granular layer cells. Irregular clusters of grey matter resembled polymicrogyric cerebellar cortex with a well--defined molecular layer. Large nests of granule cells were observed. Purkinje cells did not form a monolayer; they were irregularly dispersed in the molecular layer and among granule cell nests.
2) compact polymicrogyric pattern without well distinguishable molecular layer. Bands of granule cells or spindle cells were situated closely together, and formed a structure that corresponded to polymicrogyric cortical invaginations with a very narrow molecular layer.
3) compact, with dense composition of whirls or stripes of granule cells and spindle cells, mixed with Purkinje cells. Purkinje cells were scattered and disarranged in inappropriate places in the internal granular layer.
4) heterotopic cortex in the white matter with a normal layered structure containing all the components of the cerebellar cortex, including a normal monolayer of Purkinje cells, granule cells and molecular layer.
5) clusters of spindle cells indenting into convolutes of dentate nucleus

Malformation of the cerebellar cortex coexisting with white matter heterotopias was observed in one case. Disorganized cortical layering and gyral formation were observed in this case.

In the white matter of the cerebral hemispheres groups of neurons arrested in the course of migration were observed in the frontal, parietal, temporal and occipital lobes. The localization of arrested neurons was wide subcortical, central and subventricular in premature newborns, central in older children.

Several types of heterotopias based on their morphological picture can be distinguished:

1) widely scattered neuronal precursors from the germinal matrix formed clusters in the white matter. Great masses of heterotopic nerve tissue were located close to ventricles. A radial alignment of groups of small dark neurons in columns was evident. Young neurons formed funnel-shaped or ribbon-like structures.
2) laminar heterotopia in the form of normal immature cortex.
3) heterotopias formed by mature pyramidal neurons in disorganized, chaotic groups located in the central white matter. Neurons and glial cells created nodular heterotopias.

Coexistence with cerebral cortical malformations were observed in all cases with hemispheric white matter heterotopias. Disorganized cortical structure, sparsely cellular cortex, disturbed cortical lamination with neuronal clustering and irregular arrangement or molecular layer inside the cortical mantle, abnormal cortical layering with many waves of neurons, and polymicrogyria were observed in our cases.

Summary: The picture of clusters of neurons arrested in migration showed greatest morphological variety in the cerebellar heterotopias. The cerebral hemisphere heterotopias consisted of neuronal precursors from germinal matrix and pyramidal neurons. Pyramidal neurons did not access the final cortical position. Ectopic neurons survived in the wrong places – in subcortical white matter and close to ventricles in proliferative areas. These neurons have never migrated correctly but survived in ectopic sites and made incorrect synapses. In the cerebellar heterotopias all the cortical neurons were observed. Cells forming the external granular layer, molecular layer, internal granular layer and Purkinje neurons were present.

The consequence of arrested migration on defective cortical development, disturbed cortical layering and gyrus formation was evident in the cerebral hemispheres and minimal in the cerebellum. In the cerebral hemispheres neurons from the proliferative zone to the periventricular germinal

matrix migrate one way, along pre-formed fibres, to end up in the appropriate cortical laminar position. Due to defective neuron migration the arrest of migrating cells in the cerebral white matter leads to cortical malformations.

[A5]

(+)MK-801 and memantine do not preclude hypoxic preconditioning in a rat model of perinatal asphyxia

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To determine the role of NMDA receptors in hypoxic preconditioning in neonatal rats we studied the effects of uncompetitive NMDA receptor antagonists (+)MK-801 and memantine on the induction of tolerance to hypoxia-ischaemia by hypoxic preconditioning. Preconditioning hypoxia (7.3% O_2 at 36°C for 75 min) for 24 h preceded hypoxia-ischaemia, induced by unilateral carotid artery ligation followed by hypoxia produced as above. NMDA receptor antagonist (+)MK-801 (3 mg/kg) and memantine (5 mg/kg) were injected i.p. 1 h prior to preconditioning hypoxia. Hypoxia--ischaemia resulted in 49.7±3.2% weight deficit of the ipsilateral hemisphere, measured 14 days after the insult. Hypoxic preconditioning reduced brain damage to 33.9±3.4%. In the animals treated with (+)MK-801 or memantine instead of preconditioning the damage was diminished to 0.5±1.8% and 19.4±3.2%, respectively, while treatment with these antagonists before preconditioning resulted in similar levels of weight deficit. Weight of the control hemispheres was reduced in about 12% and 5% by pretreatment with (+)MK-801 and memantine, respectively. Hypoxic preconditioning and treatment with memantine did not influence the animal's rectal body temperature after the insult, while the application of (+)MK-801 induced a decrease in body temperature. Our results indicate that NMDA receptor antagonism does not inhibit the induction of tolerance in neonatal rats, but by itself induces a complex effect of prolonged protection against hypoxic-ischaemic brain injury combined with disturbances in brain development.

[A6]

Distribution of calcium-binding proteins and Toll-like receptors in human cerebellar pathology

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The intrauterine development of the cerebellum is most often disordered by different maternal infections whose microbial pathogens are recognized by Toll-like receptors (TLRs) and following activation of the appropriate transcription factor (such as nuclear factor--kappa B, NK-kB) the response of foetal brain parenchyma cells may be initiated. The calcium--binding proteins are a group of cytoplasmic proteins that may protect cells against injury. This group includes such proteins as calretinin (CR), calbindin D-28K (CB) and parvalbumin (PV). The distribution of all above-mentioned proteins that may protect or defend the human cerebellum against different harmful agents is not known in cerebellar pathology; thus in the present study the localization of these proteins using immunohistochemical methods was examined. Brains of 30 autopsied individuals including foetuses, children and adults with bacterial infections, cerebellar heterotopias, dysgenesias, paraneoplastic degeneration and brains of age--matched controls were used in the study. Serial sections of cerebellum were incubated in primary antibodies generated against calbindin D-28k (CB), calretinin (CR), parvalbumin (PV), GFAP, ferritin, TLR-3, TLR-4, and protein p50, a subunit of the NF-kB complex. In the cerebellum of control individuals the cell-specific distribution of CR, CB and PV was found but the TLR3 and TLR4 proteins in cerebellar cells were not present. The results of our study confirm the previous observations that all these proteins participate in development and in normal maturation of the cerebellar neurons. They suggest also that intrauterine harmful agents do not affect the content of calcium-binding proteins in neurons that at least

reach their places of destiny in dysgenetic cerebellar cortex or in those Purkinje cells that are located in heterotopias. In paraneoplastic cerebellar degeneration a diffuse loss of Purkinje cells was observed. In the cells undergoing degeneration, a progressive decrease of CB and PV was found. In two cases a variable degree of perivascular lymphocytic infiltrations and microglial nodules were found and these cells were immunopositive to TLR3 receptor protein, suggesting that the changes seen in paraneoplastic cerebellar degeneration may be due to the flare-up of a viral infection or to carcinoma-induced nutritional or metabolic imbalance.

[A7]

Pathology of vessels during prenatal development in the human brain

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The development of the blood vessels involves two main mechanisms: vasculogenesis, i.e. differentiation of endothelial cells (Ecs) *in situ* from mesenchymal precursors (angioblasts/hemangioblasts), and angiogenesis, i.e. sprouting and branching of Ecs from pre-existing vessels.

In the brains of human foetuses of 19 to 28 GW, endothelial and vascular development in the frontal lobe, temporal lobe and were studied histologically, histochemically and immunohistochemically (HαE, RCA-I, CD31, CD34), as well as ultrastructurally. The observation comprised the material from 17 foetuses after spontaneous abortion (SA).

In the SA foetal brain was observed a massive congestion of cerebral vessels and abnormalities in endothelial cells. Endothelia in the vessels of SA foetuses showed defects such as: granular structures in the cytoplasm, proliferation and paracrystalline bodies in the mitochondria and/or apoptotic changes.

Milder vascular and Ecs defects, allowing for survival, and yet observed in many nosological entities, could have been the reason for neurodevelopment abnormalities with neurodegenerative consequences, contributing to many diseases.

Abstracts
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