Blood-brain barrier breakdown and cerebellar degeneration in the course of experimental neoplastic disease. Are circulating Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) and -2α (CINC-2α) the involved mediators?

Slawomir Michalak1, Mieczyslaw Wender, Grazyna Michalowska-Wender2, Wojciech Kozubski3

1Department of Neurochemistry and Neuropathology, Chair of Neurology, Poznań University of Medical Sciences, Poland; 2Neuroimmunological Diseases Unit, Polish Academy of Sciences; 3Chair of Neurology, Poznań University of Medical Sciences, Poland

Abstract
Cerebellar degeneration belongs to indirect effects of malignancy on the nervous system. Although the involvement of immune system is accepted as a hypothesis of its pathology, the clinical observations of ineffective immunomodulatory therapy suggest complex pathomechanisms, which await elucidation. The aim of this study was to prove the blood-brain barrier integrity, its relation to cerebellar degeneration and the role of circulating Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) and Cytokine-Induced Neutrophil Chemoattractant-2α (CINC-2α) in indirect effects of experimental malignancy. Two transplantable neoplasms: breast cancer (BC) and Morris hepatoma (MH) in rats were used in the study. The blood-brain barrier breakdown was clearly proved in the course of both malignancies. We observed also morphological signs of cerebellar degeneration in both models, with linear loss of Purkinje cells and homogenization changes more pronounced in breast cancer bearing rats. We have found a significant decrease of CINC-1 concentration in serum of rats with growing MH, however BC had no effect on CINC-1 concentration. Changes in serum CINC-2α concentrations in BC did not reach the level of significance, however in MH bearing rats the concentrations increased three weeks after tumour transplantation. In conclusion, we may state that the development of cerebellar degeneration as an indirect effect of experimental neoplasm can result from blood-brain barrier (BBB) breakdown and possible passage of neurotoxic factors. The decreased serum concentration of CINC-1 as neuroprotective agent and increased CINC-2α in late stage of MH may be considered for their contribution to cerebellar degeneration.

Key words: Circulating Cytokine-Induced Neutrophil Chemoattractant-1, Circulating Cytokine-Induced Neutrophil Chemoattractant-2α, cerebellar degeneration.

Communicating author:
Slawomir Michalak, Department of Neurochemistry and Neuropathology, 49 Przybyszewskiego Str., 60-355 Poznan, phone +48 61 869 14 58, fax +48 61 869 14 44, e-mail: slamic@yahoo.com
**Introduction**

Cerebellar degeneration belongs to indirect, remote effects of malignancy, which result from tumour-host interactions. It is of high clinical significance, even if it precedes the diagnosis of primary tumour and, as such, it is related to the very early stage of neoplastic disease. Paraneoplastic cerebellar degeneration (PCD) frequently associates with breast cancer, ovarian cancer, small-cell lung cancer and Hodgkin lymphoma [40]. Immunological hypothesis of PCD emphasizes the role of autoimmune reaction against onconeural antigens with cytotoxic effects of CD8+ T cells as mediating non-apoptotic death of neurons and onconeural antibodies as bystanders [6]. The presence of cytotoxic T lymphocytes specific for the cytoplasmic antigen (PCD17/cdr2 – paraneoplastic cerebellar degeneration/cerebellar degeneration – related antigen) has been demonstrated in the blood of PCD patients [2]. Autoantibodies against cytoplasmic Purkinje cells antigen and cytotoxic lymphocytes have been induced by pcd17 CDNA in mice [38]. Onconeural antibodies associated with paraneoplastic cerebellar degeneration include anti-Yo, anti-Hu, anti-CV2/CRMP5 [31], anti-CV2 [55], anti-Tr [7], anti-Ri, anti-mGluR1 [42], anti-amphiphysin, anti-Ma and anti-VGCC [17,18,40]. Blood-brain barrier (BBB) is one of components responsible for immune privilege in the central nervous system. The integrity of blood-brain barrier remains crucial for this phenomenon in the physiological state. In experimental conditions the internalization of circulating immunoglobulins by Purkinje cells was observed in the setting of blood-brain barrier disruption [19]. Available data on the role of blood-brain barrier breakdown in the course of systemic neoplasm are currently limited to its role played in the formation of metastases. The pathology of BBB in the course of systemic neoplasm may be significant not only for the passage of immunoglobulins, but also for the penetration of neurotoxic agents associated with growing malignancy. The non-immune-mediated mechanism of cerebellar degeneration may be taken into consideration basing on clinical observations. In the case of coexisting myasthenic Lambert-Eaton syndrome and subacute paraneoplastic cerebellar degeneration an improvement of myasthenic symptoms was noticed as a result of an immunomodulating treatment [17], while the symptoms of PCD persisted. In patients with opsoclonus-myoclonus-ataxia syndrome despite successful treatment of underlying malignancy cerebellar signs persisted as well [43]. Furthermore, the remaining cerebellar signs were found [37] in 37% of anti-Yo antibody positive patients, despite a remission of the primary tumour. The experiments with passive transfer of serum immunoglobulins or mononuclear cells from peripheral blood of a patient with paraneoplastic cerebellar degeneration to mice led to the finding that the anti-Yo antibodies alone or in combination with complement or activated mononuclear cells are not the only cause of Purkinje cells loss [45]. Despite the presence of onconeural antibodies and evidence of effects of cytotoxic T cells, the pathogenesis of malignancy – associated cerebellar degeneration awaits elucidation. The clinical observations lead to the conclusion, that underlying pathomechanisms of cerebellar degeneration and the malignancy-associated immune response remain unknown. The neuropathological examination of patients with paraneoplastic cerebellar degeneration revealed atrophy, loss of Purkinje cells, reduced number of cells in granular layer, and proliferation of Bergmann glia [22]. Such signs of degeneration in cases positive for anti-Hu antibodies are associated with perivascular lymphocytic infiltrations [14]. In paraneoplastic cerebellar degeneration disintegration of Purkinje cells was associated with depletion of calbindin, that indicates important pathophysiological role of disturbances in intracellular calcium homeostasis [26]. In our previous studies on experimental neoplastic disease we have shown [32] the involvement of circulating cytokines and decreased serum insulin and thyroxin in the development of cerebellar degeneration in the course of experimental cancer.

The aim of this study was to prove a significance of blood-brain breakdown and circulating Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) and Cytokine-Induced Neutrophil Chemoattractant-2α (CINC-2α) in indirect effects of experimental malignancy on cerebellar morphology.

We used two transplantable neoplasms: breast cancer (BC) and Morris hepatoma 5123 (MH) in rats to compare the malignancy frequently associated with cerebellar degeneration (BC) and the neoplasm with no such effects observed in humans (MH). Although breast cancer-associated cerebellar degeneration was observed in anti-Yo positive patients [39], no evidence was found for the presence of onconeural antibodies in hepatoma patients. So, the two models were used to differentiate the specific and unspecific effect of neoplasms. CINC-1 and CINC-2α were selected from...
the number of factors for this study, because they express the effects both on BBB and cerebellar cells.

CINC-1 is the major chemokine, which together with the other member of the family, CINC-2α, recruits neutrophils to the central nervous system [9]. Rat CINC-1 is a counterpart of the human GRO (growth-related gene product), a member of the interleukin-8 family, and is well known as a potent chemotactic factor for rat neutrophils [49]. It is recognized as acute phase protein as well, induced by brain or peripheral tissues pathology [52] and it causes blood-brain barrier breakdown [4] and axonal damage [9]. CINC-2α shows the same neutrophil chemotactic activities as CINC-1 does [41]. GRO-beta stimulates the extracellular signal-regulated kinases in cultured cerebellar granule cells leading to enhancement of evoked and spontaneous postsynaptic currents in patch clamped Purkinje cells in the rat [35]. Those data suggest the role of GRO-beta in regulatory processes of neurotransmitter release in the cerebellum. There is also evidence for the expression of GRO-beta receptor CXCR2 in rat cerebellar granule neurones [27].

GRO-beta was found [28] to have neuroprotective effects on cerebellar granule cells. This effect was mediated through the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors. Activation of the phosphatidylinositol 3-kinase/Akt signalling pathway [46], which is involved in the inhibition of apoptosis, belongs to the effects of IL-8/CXCR2 in cerebellum [13]. GRO-beta activates sphingomyelin hydrolysis and mediates the stimulation of the stress-activated protein kinase c-Jun N-terminal kinase 1 (JNK1) [28]. Although the influence of GRO on cerebellar neurons was recognized to some extent, currently, no data are available on the effects of CINC-1 and CINC-2α on blood-brain barrier and cerebellar degeneration in experimental neoplastic disease.

Basing on previous observations, with the clinical need for elucidation of the pathomechanisms of paraneoplastic cerebellar degeneration in mind, the heretofore presented experiments were undertaken.

**Material and methods**

**The animals**

The animals used for experiments were adult female Wistar rats with breast cancer transplanted subcutaneously in the lateral thoracic region and male Buffalo rats with Morris hepatoma 5123 inoculated intramuscularly in hind limb. Intact rats of Wistar and Buffalo strain were used as controls. Experimental animals (controls and tumour bearing) originated from Department of Pathological Anatomy of Wroclaw Medical University. Breast cancer rats were sacrificed 7 and 14 days and Morris hepatoma 5123 bearing rats were sacrificed 7, 14 and 21 days after tumour transplantation under halothane anaesthesia and the carcass was perfused with a 4% neutral formalin solution. Fourteen days after breast cancer and 21 days after Morris hepatoma transplantation the experimental animals reached similar stages of neoplastic disease, with no metastases to the central nervous system. The number of animals in each studied group was ten. The experimental procedure was approved by the Ethics Committee of the Poznan University of Medical Sciences and it adhered to the guidelines of physiological society pertaining animal experimentation.

**Blood-brain barrier**

The blood-brain barrier permeability in experimental models used was tested by intraperitoneal injection of 2% Evans blue dye in 0.9% NaCl (30 mg/kg of body mass) as described previously [12]. Two hours after Evans blue administration, when rats skin, mucosa and eyes became blue the animals were sacrificed under halothane anaesthesia. One hemisphere of cerebellum was weighed and used for Evans Blue extraction in 2 ml of 100% formamide. After 18 h incubation at 37°C the homogenate was centrifuged for 15 minutes at 10 000 g and the dye concentration in supernatant was determined by light absorbance at 620 nm with a spectrophotometer (CECIL CE 1021). The Evans blue concentration in homogenate and in serum was estimated by interpolation of absorbance from a standard curve prepared for concentrations of Evans Blue ranging from 0 to 5 µg/ml. The blood-brain barrier breakdown was calculated from the following formula and expressed as fluid leakage in µl/g of tissue wet weight × h [53]:

$$\frac{\text{Evans blue (µg)/cerebellum wet weight (g)}}{\text{Evans blue serum concentration (µg/µl) × circulation time (h)}}$$

Contralateral cerebellar hemisphere was fixed in buffered formalin solution and the sections were examined under fluorescent microscope (Axioimager, Zeiss). In each tested animals 100 arteries were
analysed and the proportion of vessels presenting leakage of Evans blue was presented [%].

**Cerebellar morphometry**

Hematoxylin-eosin (H+E) and Klüver-Barrera stainings of cerebellar slices (7 µm) were used. For morphological examination of tissue slices, a JENAVAL light microscope (Carl Zeiss, Jena), and a digital camera (Canon PowerShot) were used. Morphometry was performed with the use of National Institute of Health (NIH) Image 1.34 software (http://rsb.info.nih.gov/nih-image/). The linear density of Purkinje cells (number of cells per 100 µm) was measured. Before statistical analysis, the numbers of detected Purkinje cells, were corrected by means of Abercrombie's method [1].

**Onconeural antibodies**

The presence of onconeural antibodies in each experimental animal was tested by indirect immunofluorescence. The slices of cerebellum were incubated with serum of appropriate animal and, then, with anti-rat IgG-FITC conjugates (SIGMA). As positive control we used mouse spleen sections incubated with rat anti-mouse light chains – FITC conjugated antibodies (SIGMA).

**Chemokines**

Serum CINC-1 and CINC-2α concentrations at 7th and 14th day after breast cancer transplantation and 7th, 14th, and 21st day after Morris hepatoma inoculation were measured by means of ELISA using rat assay kit (Immuno-Biological Laboratories, Aramach, Japan).

**Statistics**

Statistical analyses were performed using STATISTICA 5.0 (StatSoft Inc.) software. The distributions of all results were tested with Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilk tests. The results with normal (Gaussian) distribution were presented as mean ± standard deviation, while those manifesting non-gaussian distribution were presented in the form of median and upper-lower quartiles range. Significance of differences was tested using t-Student test in the groups of results with normal distribution and using the non-parametric Mann-Whitney test for results with non-gaussian distribution. We used analysis of variance (ANOVA) for comparison of chemokines concentrations.

**Results**

**Blood-brain barrier breakdown**

We noticed, both in breast cancer and Morris hepatoma bearing rats, the increasing leakage of fluid (Table I) in the cerebellum, basing on Evans blue measurements in tissue homogenates. Over half of arteries showed a leakage of Evans blue dye under fluorescence microscopy after one and two weeks of inoculation.

<table>
<thead>
<tr>
<th>Rats bearing</th>
<th>Control</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris hepatoma</td>
<td>41 ± 11</td>
<td>76 ± 17</td>
<td>96 ± 23</td>
<td>104 ± 27</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>32 ± 9</td>
<td>69 ± 45</td>
<td>52 ± 18</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 compared to control, †p < 0.05 comparing Morris hepatoma and breast cancer

<table>
<thead>
<tr>
<th>Rats bearing</th>
<th>Control</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris hepatoma</td>
<td>0</td>
<td>53 ± 5</td>
<td>55 ± 5</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0</td>
<td>57 ± 17</td>
<td>56 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

**p < 0.01 comparing 1st and 3rd week, ††comparing 2nd and 3rd week**
Blood-brain barrier breakdown and cerebellar degeneration

experiments (Figs. 1 and 2), when compared to controls, which did not present signs of blood-brain barrier breakdown. However, in Morris hepatoma rats, after three weeks of tumour growth the percentage of the damaged arteries was reduced comparing to first and second week (Table II). We have also observed accumulation of Evans blue dye in endothelial cells in this group of animals, which could not be noticed in other studied groups (Fig. 3).

Cerebellar morphometry

We found a significant \( (p < 0.00001) \) reduction in linear density of Purkinje cells one week (26 ± 20%) and two weeks (38 ± 22%) after breast cancer transplantation in comparison to controls (100 ± 10%). In Morris hepatoma bearing rats the progression of the disease was associated with decrease \( (p < 0.00001) \) in linear density of Purkinje cells (control – 100 ± 32%, one week – 61 ± 32%, two weeks – 55 ± 32%, and after three weeks of tumour growth – 57 ± 33%). The loss of Purkinje cells in cerebellum of Wistar rats bearing breast cancer for one week was significantly \( (p < 0.00001) \) higher than in Morris hepatoma rats at the same stage of neoplastic disease, as well as when compared to rats two weeks and three weeks after hepatoma transplantation (Table III). The rats with experimental neoplastic disease showed higher coefficients of variation \( (\nu_v) \) of Purkinje cells linear density, which indicated inhomogeneity of cellular loss (Table III). In qualitative analysis we demonstrated the neuropathological signs of cerebellar degeneration which included loss of and/or homogenization changes of Purkinje cells in H+E and Klüver-Barrera stainings (Figs. 4-7). No neutrophile or macrophages infiltration were found in cerebella of studied animals.

Onconeural antibodies

Indirect immunofluorescence did not reveal the presence of onconeural antibodies in both models of experimental neoplastic disease (Fig. 8). This observation ensured us that the abnormalities observed in cerebellum of experimental animals were independent of onconeural antibodies. A positive reaction was observed in control mouse spleen sections incu-
Fig. 4. H&E staining showing linear loss and homogenization changes of Purkinje cells in breast cancer bearing rats. Bar 50 µm.

Fig. 5. H&E staining showing linear loss of Purkinje cells in Morris hepatoma bearing rats. Bar 50 µm.

Fig. 6. Klüver-Barrera staining of cerebellum sections from breast cancer bearing rats (left) and Morris hepatoma bearing rats (right). Bar 50 µm.

Fig. 7. Klüver-Barrera staining of cerebellum sections from Morris hepatoma bearing rats. Bar 50 µm.

**Table III.** Linear density of Purkinje cells in the course of experimental disease in rats

<table>
<thead>
<tr>
<th></th>
<th>Buffalo control</th>
<th>Morris hepatoma 1 week</th>
<th>Morris hepatoma 2 weeks</th>
<th>Morris hepatoma 3 weeks</th>
<th>Wistar control</th>
<th>Breast cancer 1 week</th>
<th>Breast cancer 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje cells linear density (% of control) (mean ± SD)</td>
<td>100 ± 31</td>
<td>59*** ± 31</td>
<td>54*** ± 31</td>
<td>56*** ± 32</td>
<td>100 ± 10</td>
<td>26*** ± 20</td>
<td>39*** ± 22</td>
</tr>
<tr>
<td>$\nu_0$ (%)</td>
<td>31</td>
<td>52</td>
<td>57</td>
<td>57</td>
<td>10</td>
<td>77</td>
<td>56</td>
</tr>
</tbody>
</table>

$\nu_0$ – coefficient of variation, ***$p < 0.00001$
Blood-brain barrier breakdown and cerebellar degeneration

bated with rat anti-mouse light chains – FITC conjugated antibodies (Fig. 9).

Chemokines

CINC-1 concentration changes were insignificant in breast cancer bearing rats, while a significant decrease was noticed in Morris hepatoma animals, comparing to controls (Table IV).

CINC-2α serum concentration remained unchanged in breast cancer rats, but a significant increase was observed at 3rd weeks after Morris hepatoma inoculation (Table V).

The only significant correlation between serum chemokines level and blood-brain barrier found, was highly positive ($r = 0.89$, $p < 0.05$) for CINC-2α concentration and leakage of fluid through blood-brain barrier one week after breast cancer transplantation. We were not able to find any other correlations between serum CNC-1 and CINC-2α and the other studied parameters.

Discussion

The blood-brain barrier breakdown was clearly documented in the course of breast cancer and Morris hepatoma used in our study as experimental

**Table IV. Serum CINC-1 concentrations in breast cancer and Morris hepatoma bearing rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris hepatoma</td>
<td>328.59</td>
<td>192.84</td>
<td>290.98</td>
<td>155.44</td>
</tr>
<tr>
<td></td>
<td>27143-374.70</td>
<td>113.61-210.95$^*$</td>
<td>174.84-310.88</td>
<td>103.77-197.60$^{**}$</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>175.81</td>
<td>170.48</td>
<td>248.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>165.16-229.91</td>
<td>162.75-209.22</td>
<td>168.79-275.68</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 comparing to control, $^*$p < 0.01 comparing 2nd and 3rd week

**Table V. Serum CINC-2α concentrations in breast cancer and Morris hepatoma bearing rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris hepatoma</td>
<td>5.40</td>
<td>7.12</td>
<td>11.10</td>
<td>28.52</td>
</tr>
<tr>
<td></td>
<td>3.80-12.23</td>
<td>4.42-10.73</td>
<td>4.86-15.47</td>
<td>17.03-35.80$^{**}$</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>5.40</td>
<td>6.84</td>
<td>7.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.58-6.30</td>
<td>5.94-10.54</td>
<td>4.68-8.31</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 comparing to control, $^*$p < 0.01 comparing 1st and 3rd week, 2nd and 3rd week
models of neoplastic disease. The interplay between systemic cancer and blood-brain barrier is multilevel. Most of the studies focus on the role played by BBB breakdown in development and treatment of brain metastases [5,11,36]. Among factors taken into consideration as causes of blood-brain barrier damage in the course of cancer cytokines are the most important [15,25]. The indirect effect of systemic cancer on BBB breakdown may be mediated by number of cytokines and chemokines. However, in our study, CINC-1 was unlikley to cause such an effect in both experimental models. CINC-2α may be involved in BBB damage in the very early stage of transplantable breast development, as shown by high correlation between of CINC-2α concentration and leakage of fluid through blood-brain barrier. To our knowledge such effects were previously not shown.

There is experimental evidence on the role played by CINC-1 in neutrophil-mediated – blood-brain barrier breakdown [4]. In our study we did not noticed the neutrophile infiltrations, as well as a decrease in CINC-1 serum concentration with no relation to blood-brain breakdown. On the other hand, CINC-1 may cross BBB and when injected into blood or CSF did not caused breakdown of the BBB [34]. So in the very early stage of our model of breast cancer, it is possible, that CINC-2α causes BBB damage and enables the passage of CINC-1 to the cerebelum.

The effect of cytokines on blood-brain barrier integrity is mediated by regulation of matrix metalloproteinases. Pro-inflammatory cytokines, including interleukin-β (IL-β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interferon-γ (IFN-γ) are important regulators of expression of matrix metalloproteinases (MMP) and tissue inhibitors of matrix metalloproteinases (TIMP) [23]. IL-β and TNF-α, macrophage inflammatory protein (MIP-1α, MIP-1β, and RANTES) (regulated on activation normal T-cell expressed and secreted) up-regulate the secretion of proMMP9 by T lymphocytes, while INF-γ down-regulates it [21]. TNF up-regulates the expression of matrix metalloproteinases not only on lymphocytes T, but also on endothelial cells [21]. A particular role played by TNF as an mediator of MMP expression and synthesis is emphasized in literature [10]. On the other hand, tissue inhibitor of MMP-1 (TIMP-1) production is stimulated by IL-1β, transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF), and IL-6 [16]. TIMP-1 shows neuroprotective effect in the central nervous system by inhibiting MMPs and reducing glutamate-mediated calcium influx [44] and together with TIMP-2 inhibits apoptosis [30].

Severe blood-brain barrier breakdown noticed in our study may be one of components, which forms milieu promoting cerebellar degeneration. We have observed morphological signs of cerebellar degeneration in both models of neoplastic disease. Linear loss of Purkinje cells and homogenization changes have been more pronounced in breast cancer rats. Purkinje cells have been shown to internalize proteins from the blood and cerebospinal fluid. The leakage of neurotoxic agents through the blood-brain barrier increases, therefore, the exposition of Purkinje cells to factors, which may promote neurodegeneration.

The present study showed no effects of transplantable breast cancer on evaluated chemokines and significant decrease of CINC-1 concentration in serum of rats with growing Morris hepatoma. CINC-1 shows neuroprotective effect [48], so its depletion may promote neurodegenerative patomechanisms in cerebelum. This observation is in concordance with our previous study on Morris hepatoma effects on cytokines [32], which showed decrease in concentration of circulating tumor necrosis factor-α (TNF-α). The experimental studies showed, that local production of CINC-1 is stimulated in result of TNF-α administration [47]. Therefore depletion of TNF in the course of Morris hepatoma growth may lead to further decrease in CINC-1 concentration in serum.

We have suggested [32], that an increased local consumption of TNF-α by tumor cells is responsible for the observed decrease of its serum level. Reciprocal immunosuppression was proposed in our paper as another hypothesis of Morris hepatoma effects on TNF concentration in serum. While reactive oxygen species and nuclear factor NFκB mediate the effect of TNF on CINC-1 production [20], the role of those factors need to be taken in consideration in experimental neoplastic disease.

Other inflammatory stimuli, such as IL-β and lipopolysaccharide (LPS) may induce production of CINC-1 [33,50,51]. Recently, the induction of CINC-1 and MCP-1 in the rat brain was observed after endothelin B receptor agonist administration [24]. It needs further investigation to clarify whether those factors play significant role in the course of experimental neoplastic disease.

The stimulation of proteinase-activated receptor-2 (PAR-2) has been identified to release CINC-1 from cultured astrocytes [48]. The receptor-induced release
of CINC-1 was shown to exert neuroprotective activity, particularly preventing ceramide-induced cell death. A depletion of this circulating chemokine may, thus, limit its neuroprotective effects leading to neurodegeneration. This suggestion is supported by results of studies showing GRO-beta as a chemokine with anti-apoptotic effects mediated via glutamate AMPA receptor [29].

The induction of CINC-1 was reported as independent from CC chemokines, such as monocyte chemotactic protein-1 (MCP-1) [8]. Our previous study on circulating MCP-1 in Morris hepatoma bearing rats [32] showed increase in its serum concentration 21 days after tumour inoculation. Because NFκB influences both the production of MCP-1 and TNF-α, our previous and present studies make it unlikely that this factor is responsible for changes observed in Morris hepatoma rats. MCP-1, involved in chronic inflammatory diseases, in which monocytes/macrophages seem to be of great significance in the cytokine network, affects tumour proliferation and is involved in carcinogenesis in breast [27]. In clinical practice the increased serum MCP-1 level could be correlated with advancement of the tumour stage and lymph node involvement in patients with breast cancer. However in a mouse model of breast cancer the neoplasm responded to treatment with neutralising antibodies against MCP-1 by prolonged survival of the experimental animals and inhibition of growth of lung micrometastases [39].

The differential time-dependent production of MCP-1 and CINC-1 associates brain ischemia in rats [54] with CINC-1 increase as an early (6 hours peak) phenomenon and MCP-1 increase as a delayed (2 days peak) one. The decrease in serum CINC-1 concentration in Morris hepatoma rats may result from depletion of this chemokine in previous, early stages of tumour development. No significant changes observed in breast cancer bearing rats are probably of the same origin.

Changes in serum CINC-2α concentrations in breast cancer did not reach the level of significance, but in Morris hepatoma bearing rats it increased three weeks after tumour transplantation.

To our knowledge, no evidence has been provided on circulating CINC-2 in experimental cancer or human neoplasm until now. However, the stimulation of CINC-2 by interleukin-1β production in spinal cord and young rat brain has been already shown [9].

The absence of onconeural antibodies in our models excluded autoimmune mediated pathomechanisms of the observed cerebellar degeneration and possible effects of antibodies on stimulation of chemokines production. The induction of CINC-1 and CINC-2 may be caused by Fcγ receptors activation [3], which has not been the case in our study.

In conclusion, we may state that the development of cerebellar degeneration as an indirect effect of experimental neoplasm is rather related to blood-brain barrier breakdown and possible passage of neurotoxic factors. The decreased serum concentration of CINC-1 as a neuroprotective agent and increased CINC-2α in late stage of Morris hepatoma may be considered for their contribution to cerebellar degeneration. The evidence of cerebellar degeneration in both studied neoplasms with the absence of onconeural antibodies makes the role of cancer – associated humoral response less probable as the involved pathomechanism.

References
22. Henson RA, Urich H. Cortical cerebellar degeneration. In: Hen-

21. Hanemaaijer R, Koolwijk P, le Clercq L, de Vree WJ, van Hins-

18. Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W,

17. Graus F, Lang B, Pozo-Rosich P . P/Q type calcium channel anti-


15. Ellison MD, Povlishock JT, Merchant RE. Blood-Brain Barrier Dys-

14. Dalmau J, Graus F, Rosenblum MK, Posner JB. Anti-Hu-associa-

13. Crowder R J, Freeman RS. Phosphatidylinositol 3-kinase and Akt

12. Coyle P Different Susceptibilities to Cerebral Infarction in Spontane-


10. Chandler S, Miller KM, Clements JM, Lury J, Corkill D, Anthony DC,

9. Slawomir Michalak, Mieczyslaw Wender, Grazyna Michalowska-Wender, Wojciech Kozubski

8. Chemokine receptor expression by endothelial cells at blood-brain barrier

7. Melamed MR, Bertino JR. Multidrug-resistance gene (P-glycopro-

6. Adams SE, Gearing AJ, Matrix metalloproteinases, tumor necro-


4. Matsui H, Cepinskas G, Kvietys PR, Yoshikawa T. Tumor Necrosis

3. Pan W, Kastin AJ. Changing the chemokine gradient: CINC1

2. Koyama Y, Baba A, Matsuda T. Production of monocyte

1. Koyama Y, Baba A, Matsuda T. Production of monocyte

102

Folia Neuropathologica 2010; 48/2


