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Cachexia – induced cerebellar degeneration: Involvement of serum TNF and MCP-1 in the course of experimental neoplastic disease

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Abstract. Cerebellar degeneration may be recognized as a remote effect of a growing tumor. We have analysed serum concentrations of tumor necrosis factor-α (TNF-α), macrophage chemoattractant protein-1 (MCP-1), thyroxine and insulin to elucidate the pathomechanism which may be of importance for the development of central degeneration in cachectic Morris hepatoma bearing rats. Serum TNF-α and MCP-1 levels were evaluated by means of the ELISA system, while thyroxine and insulin were estimated by radioimmunoassay. Microscopic examination using hematoxylin-eosin, Nissl and Klüver–Barrera staining revealed an atrophy in the cerebellum, homogenization changes of Purkinje cells and decreased cell density of the granular layer. In the Morris hepatoma bearing animals serum MCP-1 content was elevated while TNF-α, thyroxine and insulin concentrations were decreased. This study has demonstrated that circulating TNF-α and MCP-1, together with decreased levels of insulin and thyroxine accompany and may produce a milieu of factors involved in mechanisms of the development of cerebellar degeneration in cachectic hepatoma bearing rats.

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Key words: cachexia, tumor necrosis factor, monocyte chemoattractant protein –1, paraneoplastic cerebellar degeneration, neurological paraneoplastic syndrome

INTRODUCTION

Cerebellar degeneration is observed in the course of neoplastic diseases and known as paraneoplastic neurological syndrome (PCD, paraneoplastic cerebellar degeneration). The syndromes are defined as non-metastatic neurological complications. The malignancies known as responsible for cerebellar degeneration include breast and ovarian tumors, small cell lung carcinoma and Hodgkin's disease (Bernal et al. 2003). Their autoimmune background is confirmed by several pieces of evidence, including antibodies identified by means of Western blotting. Paraneoplastic cerebellar degeneration patients manifest presence of anti-Yo antibodies (Peterson et al. 1992), which react with CDR-62 (Cerebellar Degeneration Related) and CDR-34 antigens. Anti-Yo antibodies react with antigens localized by means of immunoelectron microscopic analysis in ribosomes, Golgi apparatus and sarcoplasmatic reticulum of Purkinje cells. Additional antibodies identified in the patients presenting paraneoplastic cerebellar degeneration include: anti-Tr antibodies, present in Hodgkin's lymphoma (Bernal et al. 2003) and reacting with a cytoplasmic antigen, anti-MM1 antibodies reacting with nucleolar and nuclear antigens and anti-Ri antibodies reacting with neuronal cell nuclei. Anti-GAD (Glutamic Acid Decarboxylase) antibodies were also identified in patients with cerebellar degeneration (Smitt et al. 2003).

The pathomechanism of neurological paraneoplastic syndromes still needs elucidation. For the first time, Wilkinson and Żeromski (1965) identified anti-neuronal antibodies in patients with sensory neuropathy in the course of lung cancer, and this has led to an assumption that this syndrome is immunologically mediated. However, several questions remain unresolved. The first one is that some patients manifesting the paraneoplastic cerebellar degeneration are seronegative. Then, the immunosuppression as well as intravenous immunoglobulins are clinically ineffective. A very interesting case of the myasthenic Lambert–Eaton syndrome, coexisting with a subacute paraneoplastic degeneration was described by Grauss and others (2002), in which a significant improvement of myasthenic symptoms was achieved following immunomodulatory treatment while signs of subacute paraneoplastic degeneration persisted.

Morris hepatoma bearing rats may serve as an experimental model for the development of a paraneoplastic cerebellar degeneration. In this experimental model, Michalak and Adamczewska-Goncerzewicz (unpublished data) have been able to demonstrate the occurrence of a typical morphological pattern of cerebellar degeneration.

Proinflammatory cytokines, known to induce some metabolic changes which may be of importance for the development of central degeneration, were analysed in serum to elucidate the pathomechanism of the discussed syndrome.

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine which induces a variety of cellular responses in the course of neoplastic disease, including apoptosis. The mechanisms by which TNF induces apoptosis include the generation of ceramide in result of activation of acidic sphingomyelinase (ASMase) (Colell et al. 2002). Ceramide and sphingosine are lipid effectors, which play a role in neurodegenerative disorders. TNF-α activates also inflammation and immunological reactions in the brain and is known for its neurotoxic effects.

MCP-1 is a CC chemokine, attracting monocytes to the site of inflammation, which to some extent affects also T-lymphocytes, natural killer cells as well as dendritic cells.

Cytokines are also responsible for a number of cancer cachexia accompanying abnormalities. Macrophages secrete interleukin 1β (IL-1β), interleukin-6 (IL-6) and TNF-a in the course of a neoplastic disease (Meng et al. 1999).

According to some suggestions, MCP-1 may be involved in maturation of Purkinje cells, the dentate nucleus, the inferior olivary nucleus and of their interconnecting network, thus promoting growth of dendrites and synapses (Meng et al. 1999). The evidence is also available that this chemokine participates in apoptosis of both hippocampal neurons and reactive astrocytes and is capable to mediate distinct biological effects evoked by trauma and its subsequent repair (Mehta et al. 1994).

In the course of an immune response certain cytokines, such as IL-1, IL-6, and TNF-α, can signal the brain, which, through a complex corticotrophin releasing hormone-dependent pathway, triggers activation of both the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis (Elenkov et al. 2000). Both TNF-α and MCP-1 have been shown to affect endocrine system. Evidence is known for cytotoxic effect of TNF-α to pancreatic beta cells but, at the same time, this cytokine stimulated an enhanced

insulin release (Mehta et al. 1994). Moreover, TNF-α happened to exert a biphasic action on protein tyrosine kinase (PTKase) and protein-tyrosine phosphatase (PTPase) – the enzymes involved in energy metabolism, cell proliferation and stimulation of the MHC class I molecule pathway. Initially, activity of these enzymes rose but later on it was inhibited and caused insulin resistance in non-insulin dependent diabetes mellitus and in cancer (Holden et al. 1999). On the other hand, chronic administration of TNF-α decreased plasma thyroxine level (Sweep et al. 1992).

Interconnections also exist between insulin and MCP-1. Insulin has been shown to induce expression of MCP-1 in normal adipocytes (Sartipy and Loskutoff 2003).

On the other hand, both thyroid hormones and insulin produce an effect on cerebellum. In hypothyroidism the migration of granular cells is retarded (Morte et al. 2002), it leads also to reduction in arborisation (Schwartz 1983) and synaptic formation between Purkinje cells (Nicholson and Altman 1972). Insulin is essential for the culture of cerebellar cells (Huck 1983) and together with insulin – like growth factor it mediates neuroprotection (Hamabe et al. 2003).

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. The aim of this study was to prove a significance of circulating TNF-α and MCP-1 in cerebellar degeneration in cachexia, developing in experimental neoplastic disease, and to relate it to serum insulin and thyroxine levels. For this purpose, we used rats bearing transplantable Morris hepatoma, because no data are available on paraneoplastic cerebellar degeneration in the course of this type of neoplasm and we, thus, hoped to avoid possible immunologically mediated mechanisms. No evidence was found for the presence of onconeuronal antibodies in hepatoma patients, so mechanisms other than immunological ones are probable involved in those particular cases of paraneoplastic cerebellar degeneration.

METHODS

The animals used for experiments were adult male Buffalo-strain male rats, 3.5 months of age, 300–350 g of body weight. Morris hepatoma bearing rats originated from Department of Pathological Anatomy of Wroclaw Medical University where the neoplasm was transplatanted from primarily chemically induced (N-(2-fluorenyl)-phtalamic acid) tumor to next generations of experimental animals. They were injected into their left hind limbs with Morris hepatoma 5123 in the volume of 0.5 ml of hepatoma tissue. The homogenate was prepared by macroscopic dissection of hepatoma tissue and transplanted from Morris hepatoma bearing rats to experimental animals. After 21 days, the experimental rats were sacrificed under halothane anaesthesia and the carcass was perfused with a 4% neutral formalin solution. Hematoxylin-eosin, Nissl and Klüver–Barrera staining of tissue samples was used. The same procedure was used for control animals, which were male Buffalo strain rats, 3.5 months of age, 300–350 g of body weight. For morphological examination of tissue slides, a JENAVAL light microscope (Carl Zeiss, Jena) and a color Video Camera (CCD, Sony) were employed. The images were saved using the MultiScan software (Computer Scanning System 2, Poland). Morphometry was performed with the use of National Institute of Health (NIH) ImageJ 1.34 software (http://rsb.info.nih.gov/nih-image/). For the measurements of Purkinje cell number and of the thickness of granular and molecular layers six sections were selected of the same location in each animal (Fig. 1A,B). The thickness of each section was 8 mm. In twenty four fields of each section Purkinje cells were scored and in eight fields thickness of cerebellar layers was measured. The linear density, cell's area, perimeter and diameter were compared in Purkinje cells populations in controls and Morris hepatoma bearing rats. The numbers of Purkinje cells determined were corrected by means of Abercrombie's method (Abercrombie 1946) before statistical analysis. Furthermore, a particular attention was paid for staining quality of Purkinje cells and differentiation between unstained cells and free space remaining after cells loss. Measurements of linear density as well as area, perimeter and diameter were performed in sections stained with hematoxylin-eosin, and Nissl and Klüver–Barrera staining. Unstained Purkinje cells, if present, show a contour which enabled measurements. In result of cell loss there were cellular residues present indicating former Purkinje cells.

Blood samples were obtained by heart puncture and in the serum thereof TNF-α and MCP-1 levels were quantified by means of the ELISA system (Quantikine rat, R&D systems, USA). Thyroxine and insulin levels were determined by means of radioimmunoassay (RIA, LKB Wallac 1275 Minigamma Counter).

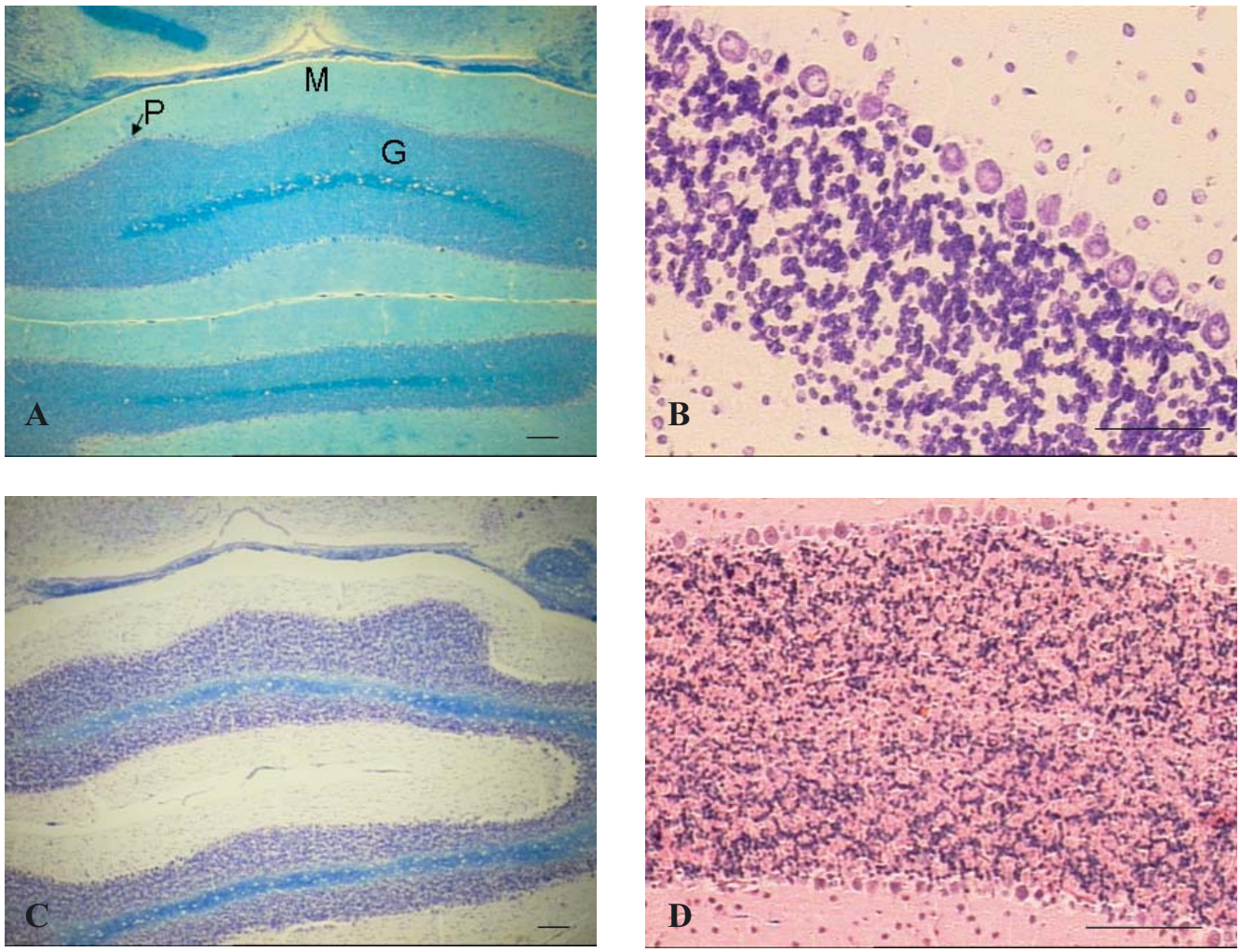

Fig. 1. Hemisphere of the cerebellum – Morris hepatoma bearing rat. (A) Klüver–Barrera; (B) (H+E) staining. Controls: (C) Klüver–Barrera; (D) (H+E) staining. Scale bars are 100 mm. (P) Purkinje cell; (G) granular layer; (M) molecular layer. Arrows: (···) homogenization changes in Purkinje cell; (---) linear loss of Purkinje cells.

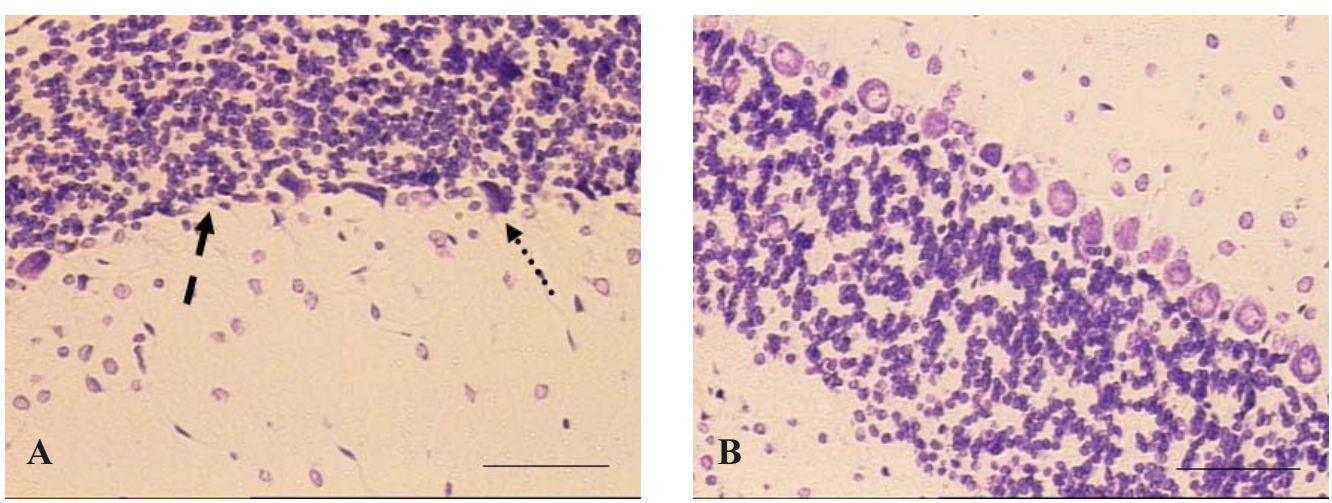

Fig. 2. Hemisphere of the cerebellum. (A) Morris hepatoma bearing rat; (B) controls (Nissl staining). Scale bars are 100 mm. Arrows: (···) homogenization changes in Purkinje cell; (---) linear loss of Purkinje cells.

The number of animals in both experimental and control groups was ten. 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. Significance of differences was tested using Student-t test in the groups of results with normal distribution and using the non-parametric Mann-Whitney test for results with non-gaussian distribution.

The experimental procedure was approved by the Ethics Committee of the University of Medical Sciences and it adhered to the guidelines of physiological society pertaining animal experimentation.

RESULTS

On the 21st day after inoculation of the Morris hepatoma 5123, body mass of the experimental animals was reduced to 67 ± 7% (x ± SD) of the baseline value. Thus, the tumor bearing animals were obviously cachectic and presented a decreased locomotor activity, paresis of limbs and balance disturbances.

Microscopical examination of the transplanted tumor revealed an active neoplastic process with numerous atypical cells and with necrotic changes.

In the cerebellum of hepatoma bearing rats, stained with H+E and Klüver–Barrera, a linear loss of Purkinje cells was seen in the hemispheres (Fig. 1A,B), with spared cells in the vermis. Furthermore, cell density in the granular layer was decreased (Fig. 1B) and homogenization changes in Purkinje cells were evident (Fig. 1A,B). Also, Nissl staining revealed a loss of Purkinje cells and homogenization changes (Fig. 2A). The quantitative morphometry revealed decreased (P<0.0001) number of Purkinje cells in vermis and cerebellar hemispheres of rats with growing tumor (43 ± 21% and 63 ± 35%, respectively, as compared to control) (Fig. 3). The thickness of granular layer was significantly decreased both in vermis and in cerebellar hemispheres (P<0.001 and P<0.0001, respectively) of experimental animals (84 ± 28% and 75 ± 23%, respectively) (Fig. 3). However, the thickness of molecular layer was increased in vermis of cachectic rats and remained

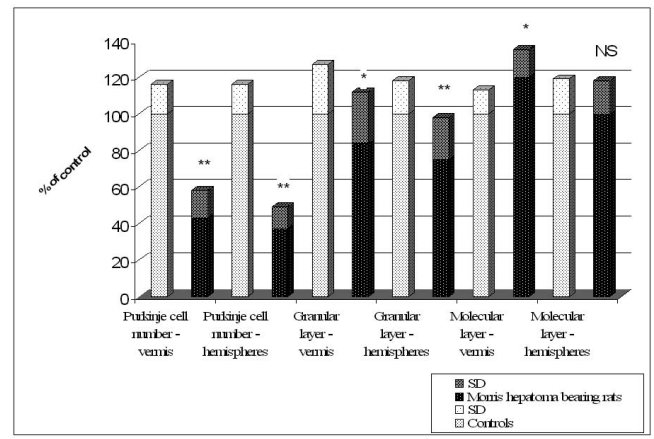

Fig. 3. Purkinje cell number, granular and molecular layers thickness in vermis and cerebellar hemispheres in Morris hepatoma bearing rats. (*) P<0.001; (**) P<0.0001; (NS) statistically non-significant.

intact in hemispheres. The areas of Purkinje cells in vermis and hemispheres were reduced in neoplastic rats (65 ± 32% and 79 ± 27% , respectively, when compared to control, P<0.001) (Figs 4 and 5), the same was for perimeter (80 ± 17% and 90 ± 16%, respectively, when compared to control, P<0.001) (Figs 6 and 7) as well as for diameter (77 ± 17% and 81 ± 24%, respectively, comparing to control, P<0.001) (Figs 8 and 9). Hence, morphological features of cerebellar degeneration could be proved in the Morris hepatoma bearing rats, as compared to controls (Figs 1C,D and 2B).

The concentration of MCP-1 in serum was significantly (P<0.01) increased in cachectic rats (median: 1422.62 pg/mL; lower–upper quartile: 1194.77–1743.42 pg/mL). The serum concentration of MCP-1 in control animals was 882.35 pg/mL (median; lower–upper quartile: 828.57–1220.77 pg/mL) (Table I).

There was a significant (P<0.001) decrease of TNF-α content in the serum of rats with morphological signs of cerebellar degeneration and cachexia (median: 28.7 pg/mL; lower–upper quartile: 23.2–35.6 pg/mL).The controls presented higher serum concentration of TNF-α (55.8 pg/mL – median; lower–upper quartile: 48–106 pg/mL) (Table I).

A decrease in serum thyroxine concentration (7.01 ± 0.57 μg/dL, P<0.05) was observed, as compared to control values (9.02 ± 0.45 μg/dL). Likewise, serum insulin concentration was reduced in the tumor bearing animals (1.05 ± 0.04 μIU/mL, P< 0.05) compared to that of controls (3.17 ± 0.07 μIU/mL) (Table I).

Table I

Serum concentrations of MCP-1, TNF-α, thyroxine, and insulin in Morris hepatoma bearing rats (n=10, age 3.5 months) and controls (n=10, age 3.5 months)

	MCP-1 pg/mL	TNF- α pg/mL	Thyroxine μg/dL	Insulin μIU/mL
Controls	882.35 (828.57–1220.77)	55.8 (48–106)	9.02 ± 0.45	3.17 ± 0.07
Morris hepatoma bearing rats	1422.62 ** (1194.77–1743.42)	28.7 *** (23.2–35.6)	7.01 ± 0.57 *	1.05 ± 0.04 *

The values for MCP-1 and TNF-α are median and lower-upper quartile values while those for thyroxine and insulin are means ± standard deviations

DISCUSSION

The pathogenesis of the paraneoplastic cerebellar degeneration is as yet not fully understood. The possibilities include autoimmune reaction and metabolic changes in the nervous system evoked by the tumor itself and the subsequent cachexia. Neuropathological findings presented in literature and corresponding to paraneoplastic cerebellar degeneration include loss of Purkinje cells, neuronal loss, demyelination in the dentate nucleus and thinning of the granular and molecular layers. The white matter shows signs of secondary degeneration in which the deep cerebellar nuclei are only rarely involved (Brain and Wilkinson 1965, Vick et al. 1969). These morphological features are accompanied by perivascular lymphocytic infiltrations and by loss of neurons in the brainstem and spinal cord. In most cases no inflammatory reaction is present (Brain and Wilkinson 1965, Vick et al. 1969). A considerable loss of Purkinje cells, proliferation of the Bergmann glia and neuronal loss in the dentate nucleus and in the lower olives, without any appreciable inflammatory reaction was noticed in light microscopy by Bernal and coauthors (2003), who examined patients with anti-Tr antibodies. Loss of Purkinje cells and reduction of the cell density in the granular layer are the morphological findings made also in our study, thus indicating a cerebellar degeneration developing in Morris hepatoma bearing cachectic rats.

It is commonly acknowledged that some cytokines exert a considerable effect on tumor growth, which may be evaluated by measuring changes of their serum concentration. TNF-α is one of important multifunctional cytokines involved in the effects of malignancies but its role in development of cerebellar degeneration is so far not resolved. Evidence has been shown that TNF-α reduces the capacity of the insulin-like growth factor I (IGF-I) to promote the survival of primary murine neurons in the cerebellar granular layer (Venters et al. 1999). Furthermore, TNF-α concentrations correlate positively with the extent and severity of the disease in patients with prostate cancer (Michalaki et al. 2004); increased serum concentrations being also associated with the progress of the disease and malnutrition in hepatocellular carcinoma. Mantovani and others (2001), however, were unable to demonstrate a statistically significant increase in the serum TNF-α level in cachectic persons. Catalano and colleagues (2003) have established that TNF-α is increased in the brain and in other organs of tumor bearing cachectic rats but no TNF-α has been found in sera of the animals. In view of these observations, our findings showing a diminished concentration of TNF-α in serum of Morris hepatoma bearing rats are by no means surprising. It may be assumed that an increased local consumption of TNF-α by tumor cells is responsible for the observed decrease of its serum level. Another hypothesis, which cannot be excluded, allows for a possible effect of reciprocal immunosuppresion.

MCP-1 is one among other proinflammatory cytokines, which seems to be of great significance in the cytokine network, affecting tumor proliferation and involved in breast carcinogenesis (Lebrecht et al. 2004). In patients with breast cancer, the increased serum MCP-1 level could be correlated with advancement of the tumor stage and lymph node involvement. In a mouse model, cells of human breast cancer responded to treatment with neutralising antibodies

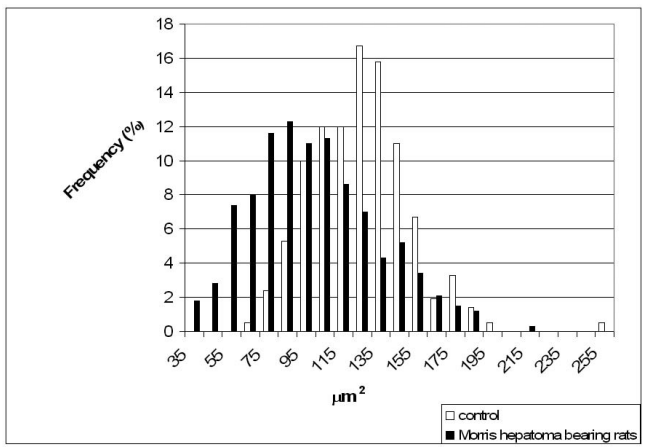

Fig. 4. Histograms of areas of Purkinje cells in cerebellar hemispheres (μm^2)

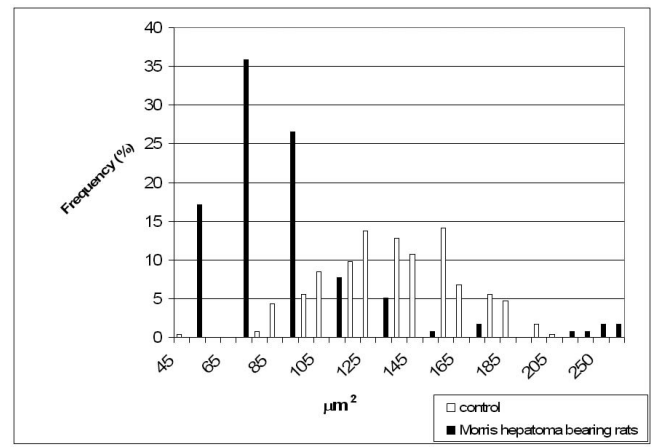

Fig. 5. Histogram of areas of Purkinje cells in cerebellar vermis (μm^2)

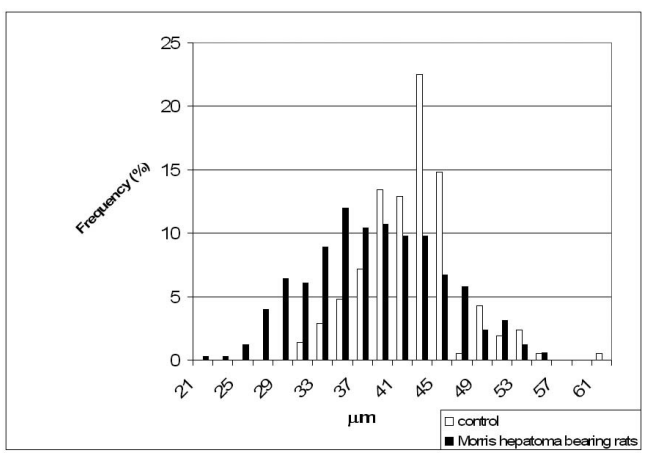

Fig. 6. Histogram of perimeters of Purkinje cells in cerebellar hemispheres (μm)

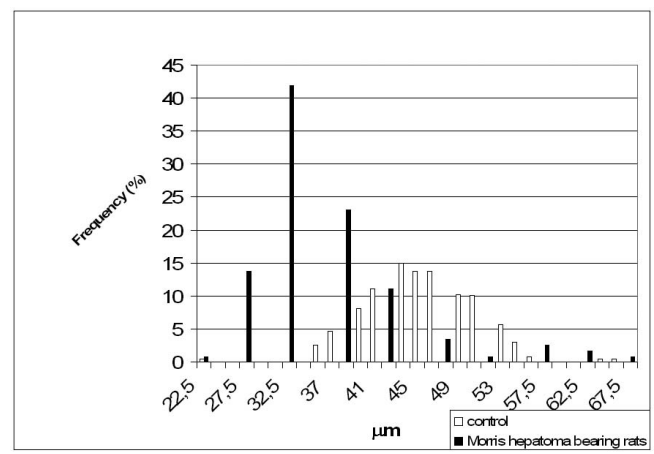

Fig. 7. Histogram of perimeters of Purkinje cells in cerebellar vermis (μm)

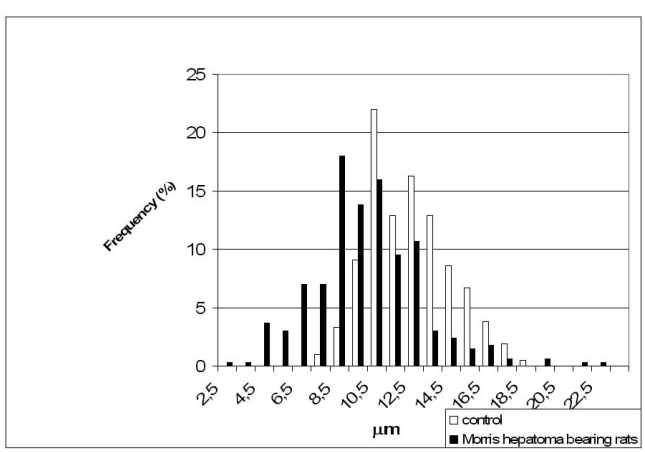

Fig. 8. Histogram of diameters of Purkinje cells in cerebellar hemispheres (μm)

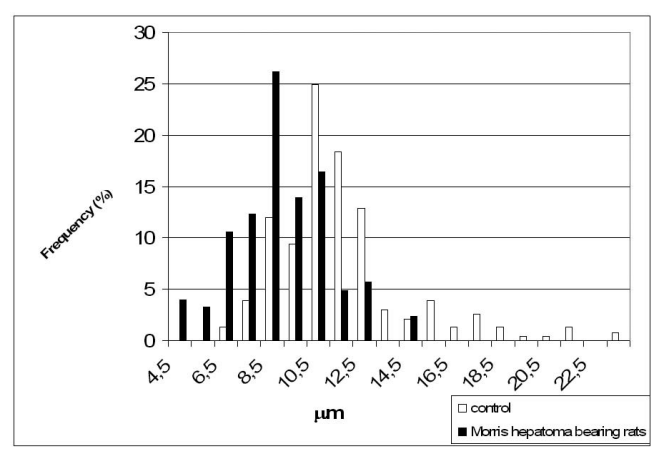

Fig. 9. Histogram of diameters of Purkinje cells in cerebellar vermis (μm)

against MCP-1 by elongated survival of the experimental animals and inhibition of growth of lung micrometastases (Salcedo et al. 2000). In patients with brain tumors, MCP-1 concentration in the cerebrospinal fluid served as a reliable predictor of malignancy (Kuratsu et al. 1993). From the observations of Tonouchi and coauthors (2002) it appears evident that serum level of MCP-1 decreases when gastric cancer is in progress, which also would corroborate the hypothesis of local consumption of the cytokine.

In our study, a significant increase of MCP-1 expression has been evident in serum of the cachectic rats. However, we have been unable to detect any monocyte or other mononuclear infiltrates in the central nervous system.

There are three known routes by which peripheral cytokines, including TNF-α and MCP-1, can affect the brain metabolism (Licinio and Wong 1997) and they include peripheral nerves themselves, brain vasculature, which can convey signals through secondary messengers such a nitric oxide or prostenoids and, lastly, a direct effect imposed after crossing the blood-brain barrier. MCP-1 is also known as an important factor in the cytokine network which regulates tumor cell proliferation as well as induces development of cancer cachexia. The lack of cellular infiltrations in the degenerating central nervous system in the presented experimental paraneoplastic model cannot exclude completely the involvement of an immunological reaction in the pathomechanism of the described events. However, it seems to indicate a probable effect of metabolic changes, which might have led to the development of subacute cerebellar degeneration. In addition, insulin and TNF-α have been shown to be important factors which may be involved in vivo in the progression of metastatic breast cancer (Bozcuk et al. 2004). The two factors interact in such a way that lower levels of TNF-α increase insulin secretion while higher ones happen to inhibit it (Holden et al. 1999). In the presented study, we have found decreased levels of both TNF-a and insulin in rats with cerebellar degeneration following development of cachexia. Contrary to the above mentioned observation (Sweep et al. 1992), the decrease in serum thyroxine levels in our experimental animals could probably be associated with lowered TNF-α concentration because of the prolonged (three weeks) course of the neoplastic disease. Furthermore, thyroid hormones were shown to be involved in Purkinje cells development, differentiation and dendritogenesis by acting on specific receptors (Heuer and Mason 2003, Morte et al. 2002). Thyroid hormone acts on the cerebellar cortex, particularly on Purkinje cells *via* up-regulation of G protein gene expression (Alvarez et al. 2005) and by increasing neurotrophin–3 levels (Neveu and Arenas 1996). Neuroprotective effects of insulin were found to be mediated by protein kinase C activation (Hamabe et al. 2003).

The interpretation that the changes in the levels of TNF, MCP-1, insulin and thyroxine are the results independent of the tumor growth should be taken in consideration. Nevertheless, we are of the opinion that they built the milieu of factors enabling cerebellar degeneration. MCP-1 is known (Stamatovic et al. 2005) to increase permeability of blood–brain barrier and, even if not involved by itself in cerebellar degeneration, it may lead to increased penetration of different factors to the central nervous system.

CONCLUSION

We may state that we have been able to demonstrate that the circulating TNF-α and MCP-1 together with the decreased serum levels of insulin and of thyroxine accompany and participate in the mechanism of the development of paraneoplastic cerebellar degeneration (PCD) in cachectic Morris hepatoma bearing rats.

REFERENCES

Abercrombie M (1946) Estimation of nuclear population from microtome sections. Anat Rec 94: 239–247.

Alvarez M, Perez-Castillo A, Santos A (2005) Thyroid hormone regulates Gai1 gene expression in the rat cerebellar cortex during post-natal development. J Neurochem 92: 395–404.

Bernal F, Shams'ili S, Rojas I, Sanchez-Valle R, Saiz A, Dalmau J, Honnorat J, Smitt PS, Grauss F (2003) Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. Neurology 60: 230–234.

Bozcuk H, Uslu G, Samur M, Yildiz M, Őzben T, Őzdogan M, Artac M, Altunbas H, Akan I, Savas B (2004) Tumour necrosis factor-alpha, interleukin-6, and fasting serum insulin correlate with clinical outcome in metastatic breast cancer patients treated with chemotherapy. Cytokine 27: 58–65.

Brain L, Wilkinson M (1965) Subacute cerebellar degeneration associated with neoplasms. Brain 88: 465–478.

Catalano MG, Fortunati N, Arena K, Costelli P, Aragno M, Danni O, Boccuzzi G (2003) Selective up-regulation of tumor necrosis factor receptor I in tumor-bearing rats with cancer-related cachexia. Int J Oncol 23: 429–436.

Colell A, Morales A, Fernandez-Checa JC, Garcia-Ruiz C (2002) Ceramide generated by acidic sphingomyelinase contributes to tumor necrosis factor-K-mediated apoptosis in human colon HT-29 cells through glycosphingolipids formation. Possible role of ganglioside GD3. FEBS Lett 526: 135–141.

Elenkov IJ, Wilder RL, Chrousos GP, ViziI ES (2000) The sympathetic nerve – an integrative interface between two supersystems: The brain and the immune system. Pharmacol Rev 52: 595–638.

Grauss F, Lang B, Pozo-Rosich P, Saiz A, Casamitjana R, Vincent A (2002) P/Q type calcium channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. Neurology 59: 764–766.

Hamabe W, Fujita R, Ueda H (2003) Neuronal necrosis inhibition by insulin through protein kinase C activation. J Pharm Exp Ther 307: 205–212.

Heimdal JH, Aarstad HJ, Klementsen B, Olofsson J (1999) Ex vivo interleukin (IL)-1b, IL-6, IL-12 and tumor necrosis factor-a responsivness with monocytes from patients with head and neck carcinoma. Eur Arch Otorhinolaryngol 256: 250–256.

Heuer H, Mason CA (2003) Thyroid hormone induces cerebellar Purkinje cell dendritic development via the thyroid hormone receptor a1. J Neurosci 23: 10604–10612.

Holden RJ, Pakula IS, Mooney PA (1999) Tumor necrosis factor-a: A continuum of liability between insulin-dependent diabetes mellitus, non-insulindependent diabetes mellitus and carcinoma. Med Hypotheses 52: 319–323.

Huck S (1983) Serum-free medium for cultures of the postnatal mouse cerebellum: Only insulin is essential. Brain Res Bull 10: 667–674.

Kalehua AN, Nagel JE, Whelchel LM, Gides JJ, Pyle RS, Smith RJ, Kusiak JW, Taub DD (2004) Monocyte chemoattractant protein-1 and macrophage inflammatory protein-2 are involved in both excitotoxin-induced neurodegeneration and regeneration. Exp Cell Res 297: 197–211.

Kuratsu J, Yoshizato K, Yoshimura T, Leonard EJ, Takeshima H, Ushio Y (1993) Quantitative study of monocyte chemoattractant protein-1 (MCP-1) in cerebrospinal fluid and cyst fluid from patients with malignant glioma. J Natl Cancer Inst 85: 1836–1839.

Lebrecht A, Grimm C, Lantzsch T, Ludwig E, Hefler L, Ulbrich E, Koelbl H (2004)Monocyte chemoattractant protein-1 serum levels in patients with breast cancer. Tumour Biol 25: 14–17.

Licinio J, Wong ML (1997) Perspectives series: Cytokines and the brain. J Clin Invest 100: 2941–2947.

Mantovani G, Macci A, Madeddu C, Mura L, Massa E, Mudu MC, Mulas C, Lusso MR, Gramignano G, Bonaria Piras M (2001) Serum values of proinflammatory cytokines are inversely correlated with serum leptin levels in patients with advanced stage cancer at different sites. J Mol Med 79: 406–414.

Mehta VK, Hao W, Brooks-Worrell BM, Palmer JP (1994) Low-dose interleukin 1 and tumor necrosis factor individually stimulate insulin release but in combination cause suppression. Eur J Endocrinol 130: 208–214.

Meng SZ, Oka A, Takashima S (1999) Developmental expression of monocyte chemoattractant protein-1 in the human cerebellum and brainstem. Brain Dev 21: 30–35.

Michalaki V, Syrigos K, Charles P, Waxman J (2004) Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer 90: 2312–2316.

Morte B, Manzano J, Scanlan T, Vennstrom B, Bernal J (2002) Deletion of the thyroid hormone receptor a1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci U S A 99: 3985–3989.

Nicholson JL, Altman J (1972) Synaptogenesis in the rat cerebellum: Effects of early hypo- and hyperthyroidism. Science 176: 530–532.

Neveu I, Arenas E (1996) Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. J Cell Biol 133: 631–646.

Peterson K, Rosenblum MK, Kotanides H, Posner JB (1992) Paraneoplastic cerebellar degeneration.I. A clinical analysis of 55 anti-Yo antibody-positive patients. Neurology 42: 1931–1937.

Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ (2000) Human endothelial cells express CCR2 and respond to MCP-1: Direct role of MCP-1 in angiogenesis and tumor progression. Blood 96: 34–40.

Sartipy P, Loskutoff DJ (2003) Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci U S A 100: 7265–7270.

Schwartz H (1983) Effect of thyroid hormone in growth and development. In: Molecular Basis of Thyroid Hormone Action (Oppenheimer J, Samuels H, eds). Academic Press, New York, p. 413–444.

Smitt PS, Kinoshita A, De Leeuw B, Moll W, Coesmans M, Jaarsma D, Henzen-Longmans S, Vecht Ch, De Zeeuw Ch, Sekiyama N, Nakanishi S, Shigemoto R (2003) Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. N Engl J Med 342: 21–27.

Stamatovic SM, Shakui P, Keep RF, More BB, Kunkel SL, Van Rooijen N. Andjelkovic AV (2005) Monocyte chemoattractant protein-1 regulation of blood–brain barrier permeability.J Cereb Blood Flow Metab 25: 593–606.

Sweep CG, van der Meer MJ, Ross HA, Vranck R, Visser TJ, Hermus AR (1992) Chronic infusion of TNF-alpha reduces plasma T4 binding without affecting pituitary-thyroid activity in rats. Am J Physiol 263: E1099–105.

Tonouchi H, Miki C, Tanaka K, Kusunoki M (2002) Profile of monocyte chemoattractant protein-1 circulating levels in gastric cancer patients. Scand J Gastroenterol 37: 830–833.

Venters HD, Tang Q, Liu Q, VanHoy RW, Dantzer R, Kelley KW (1999) A new mechanism of neurodegeneration: A proinflammatory cytokine inhibits receptor signaling by a survival peptide. Proc Natl Acad Sci U S A 96: 9879–9884.

Vick N, Schulman S, Dau P (1969) Carcinomatous cerebellar degeneration: Encephalomyelitis and sensory neuropathy (radiculitis). Neurology 19: 425–441.

Wilkinson PC, Żeromski J (1965) Immunofluorescent detection of antibodies against neurones in sensory carcinomatous neuropathy. Brain 88: 529–583.

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