ANTICANCER RESEARCH *37*: 5777-5783 (2017)
doi:10.21873/anticanres.12019

Association Between Interleukin-10 Receptors and the CD45-Immunophenotype of Central Nervous System Tumors: A Preliminary Study

LUKASZ ZADKA[1,2], PAWEL KRAM[3], JEREMI KOSCINSKI[4], ROMAN JANKOWSKI[4], MARIUSZ KACZMAREK[5], KATARZYNA PIATEK[6], MICHAL KULUS[1], AGNIESZKA GOMUŁKIEWICZ[1], ALEKSANDRA PIOTROWSKA[1] and PIOTR DZIEGIEL[1,7]

[1]*Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland;*
[2]*II Department of Psychiatry and Psychiatric Rehabilitation, Medical University of Lublin, Lublin, Poland;*
[3]*Department of Neurology and Cerebrovascular Disorders, L. Bierkowski Hospital, Poznan University of Medical Sciences, Poznan, Poland;*
[4]*Department of Neurosurgery and Neurotraumatology, Poznan University of Medical Sciences, Poznan, Poland;*
[5]*Department of Immunology, Poznan University of Medical Sciences, Poznan, Poland;*
[6]*Department of Endocrinology, Metabolism and Internal Diseases, Poznan University of Medical Science, Poznan, Poland;*
[7]*Department of Physiotherapy, University School of Physical Education, Wroclaw, Poland*

Abstract. *Background/Aim: One of the current hypotheses assumes that brain tumors exert an immunosuppressive influence on the surrounding cellular environment. Interleukin-10 (IL-10) is one of the immunosuppressive cytokines modifying the biological activity of cancer. The aim of this study was to assess the expression of IL10R in CD45$^+$ cells within primary brain tumors and metastases and establish its association with tumor basic immunophenotype. Patients and Methods: Tissue samples were obtained intraoperatively during surgeries of 32 patients suffering from meningiomas (n=9), gliomas (n=12) and metastatic tumors (n=11). Expression was assessed with flow cytometry and immunohistochemical reactions. Results: Expression of IL10R subunits within the leukocyte population (CD45$^+$ cells) was significantly higher in primary tumors than in metastases. Conclusion: To the best of our knowledge, this is the first study describing a correlation between the IL10R expression on leukocytes and histological types of brain tumors.*

Primary brain tumors are a heterogeneous group of neoplasms of meninges and neuroglial tissue with varying degrees of biological malignancy. Almost half of all primary tumors are gliomas and meningiomas (1). The second, which arise from arachnoid granulations, is the most frequently occurring benign brain tumor (2). Nevertheless, the most common neoplastic lesions within the brain are metastases (3). The biochemical structure of these tumors varies greatly and the assessment of their complicated molecular phenotype requires the application of sophisticated diagnostic tools (4). A considerable diagnostic problem may be caused by the fact that brain tumors tend to have parts with different tendency to metastasis. The tissue samples obtained intraoperatively are frequently small and may not reflect the morphology of tumor and its microenvironment. Tomita *et al*. compared the results of surgical biopsies and autopsies and found considerable discrepancies between their results, concluding that biopsy has little prognostic value (5).

The immune system may play a key role in the pathogenesis of brain tumors, and this putative influence is currently being widely investigated (6-7). The activity of microglial cells, macrophages and T lymphocytes through cytokine synthesis may have a profound influence on brain tumor malignancy (8). Lack of balance between the activity of T-helper (Th1 and Th2) lymphocytes was reported within meningiomas and glial tumors, which led to elevated IL-10 levels in blood serum (9).

IL-10 as an immunosuppressive cytokine affects Th1 cells. It limits the activity of phagocytes by inhibiting the secretion of proinflammatory proteins, and its ability to silence the expression of MHC class II molecules and to lower the intracellular concentrations of peroxide anions, including nitrogen oxide (NO) (10).

Correspondence to: Łukasz Zadka, Department of Histology and Embryology, Wroclaw Medical University, Wrocław, Poland. Tel: +48 717841682, e-mail: ceadr7@gmail.com

Key Words: Leukocytes, IL10RA, IL10RB, interleukins, brain tumors.

Table I. *Clinicopathological data*.

	Meningioma	Glioma	Metastases
Avg. age (years)±SD, range (years)	53±14.38, 35-75	59.42±7.66, 45-75	59.82±10.90, 38-78
Gender (M:F)	3:6	7:5	5:6
Diagnosis (n)	Atypical meningioma (2); lymphoplasmacyte-rich meningioma/ atypical meningioma (1); meningioma (indefinite) (2); fibrous meningioma (2); angiomatous meningioma partial clear cell meningioma (1); meningothelial meningioma partial psammomatous (1);	Glioblastoma (7); astrocytoma anaplasticum (2); gliosarcoma (2); neuroepithelial tumor (1) *	Suspected origin Lung (1) Unknown (9) Breast (1)

*According to the 2016 World Health Organization Classification of Tumors of the Central Nervous System Dysembryoplastic neuroepithelial tumor belongs to mixed neuronal-glial tumors. M: Male; F: female.

Furthermore, the lack of balance between Th1 and Th2 cytokines among neurooncological patients can be explained by the susceptibility of regulatory T lymphocytes (Tregs) to induction of immunosuppression as a result of elevated IL-10 synthesis (11). An intensified IL-10 production by Tregs may exert an immunosuppressive effect on macrophages and thus inhibit proinflammatory Th1 cytokine synthesis and limit the ability of antigen presentation of dendritic cells to cytotoxic lymphocytes (12).

The *in vitro* influence of IL-10 resulted in a decreased proliferation of naive $CD4^+$ lymphocytes and cytotoxic T cells (13). IL-10 was reported to substantially increase the cytotoxicity of $CD8^+$ T lymphocytes and therefore it seems to refute the potential anticancer properties of this cytokine (14) but other publications indicate on suppressive effect of IL-10 on $CD8^+$ lymphocytes (15). Nevertheless, the repeatedly obtained overexpression of IL-10 in malignant gliomas seems to confirm the importance of T lymphocytes in brain tumor pathogenesis (16, 17).

Pleiotropic influence of IL-10 on the cellular biology is exerted through its interactive contact with the membrane receptor complex. Structurally, IL10R is a tetramer composed of two α and two β subunits. The α subunit (IL10Rα) has a stronger affinity to IL-10, and it occurs only within IL10R, whereas IL10Rβ is also an integral part of other receptors of the IL-10 family of cytokines (10, 18-20). The bonding of IL-10 with the extracellular ligand-binding domains leads to the activation of JAK1 and TYK2 kinase pathways and STAT3 transcription factor (21). Although the intracellular IL10R signaling pathway has been widely described, its significance when it comes to regulating cellular proliferation and apoptosis in neoplastic processes has nonetheless not been fully elucidated (22).

The resent preliminary study is aimed to find correlation between expression of IL10R in $CD45^+$ cells in different brain tumor types.

Materials and Methods

Patients. The study included patients that were operated on in the Department of Neurosurgery and Neurotraumatology in Poznan between 2010 and 2012 (Table I). Inclusion criteria were as follows: age greater than 18 years and appropriate histological diagnosis: gliomas (n=12), meningiomas (n=9) and metastases (n=11). All metastases were thought to be primary tumors during diagnostic imaging and most of them were of unknown origin. Due to small size and heterogeneity of groups, this study should be considered as preliminary.

Informed consent from all participating patients and approval of the local bioethical committee were obtained (Committee on Bioethics, Poznan University of Medical, resolution no. 304/11). All methods were carried out in accordance with the relevant guidelines and regulations. All experimental protocols were approved by a licensing committee.

Brain tumor tissues were sampled intraoperatively during their surgical resection. Subsequently, the samples were transported to the Department of Immunology of Chair of Clinical Immunology at Poznan University of Medical Sciences. After preserving a part of samples by deep-freezing (T=80˚C), cell suspensions were obtained by mechanical grinding of the samples on Petri dishes (homogenization). All the patients included in this study were treated with dexamethasone (8 mg per day) as means of preventing cerebral edema.

Flow cytometry. In order to determine the immunophenotype of leukocytes within the inflammatory infiltration of brain tumors flow cytometry was used. Within the study we investigated CD45 positive cells, *i.e.* leukocytes. The expression of membrane receptors IL10Rα and IL10Rβ was assessed as the immunophenotype of the infiltrating leukocytes. Monoclonal

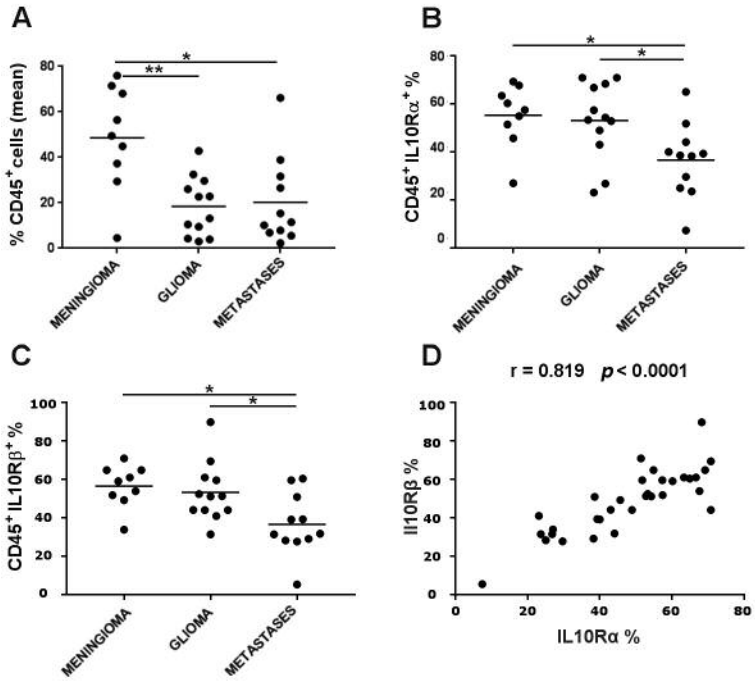

Figure 1. *Statistical analysis of flow cytometry results. A: Percentage of CD45-positive cells in different tumor types, B: percentage of CD45/IL10Rα and C: CD45/IL10Rβ-positive cells in different tumor types. D: Correlation between expression of IL10Rα and IL10Rβ on analyzed brain tumors. *p<0.05, **p<0.01.*

antibodies conjugated with appropriate flourochromes were used, directed against receptors for the following antigens: CD45 (348815, BD Biosciences, San Jose, CA, USA), IL10Rα (FAB274P, R&DSystems, Minneapolis, MN, USA) and IL10Rβ (FAB874P, R&DSystems, Minneapolis, MN, USA). Cell suspensions obtained during mechanical dissociation of the tumors were incubated with appropriate antibodies for 15 min in darkness at room temperature. Erythrocyte lysis was triggered by a lysis buffer for 10 min in darkness at room temperature. Unbound antibodies, platelets, lysed erythrocytes, other proteins and morphotic elements were discarded from the mixture by means of double centrifugation in phosphate buffer (PBS). Data acquisition of the prepared cell suspensions was conducted using 6-color flow cytometry FACSCanto (BD Biosciences, San Jose, CA, USA). Obtained results were analyzed using FACS Diva software (BD Biosciences, San Jose, CA, USA).

Slides preparation on cryostat. Frozen parts of tumors were cut into histological slides with Leica CM 1950 Cryostat. Tumors were embedded in Optimal Cutting Temperature compound (OCT) and then cut into 8 μm slides in –20˚C. These slides were used to perform immunohistochemistry (IHC) and immunohistofluorescence (IHF).

Immunohistochemistry. IHC reaction was performed on 8 μm slides prepared on a cryostat. Slides were air-dried overnight. Initially, endogenous peroxidase was blocked with Envision Flex Peroxidase-Blocking Reagent (Dako, Hamburg, Germany, 10 min). Mouse monoclonal antibody against CD45 (Dako, Hamburg, Germany, ref. M0701), were used as primary antibodies (1:50 in EnVision Flex Antibody Diluent, 20 min). Then slides were incubated in EnVision FLEX/HRP (20 min). Reaction was visualized with freshly prepared 3,3'-diaminobenzidine (DAB) (5 min). Slides were stained additionally with EnVision Flex Hematoxylin (5 min). After IHC reaction and staining, slides were dehydrated in ethanol (70%, 96%, absolute, 5 min) and xylene (5 min), then closed with Dako Mounting Medium.

IHC reaction was performed on Dako Autostainer Link48. Slides were evaluated under Olympus BX41 light microscope.

Immunohistofluorescence. IHF reaction was performed on 8-μm slides prepared using a cryostat. Slides were air-dried overnight and fixed in 4% paraformaldehyde (12 min). After a PBS wash, slides were incubated overnight with primary monoclonal mouse antibodies against CD45 (Dako, ref. M0701, 1:100 in EnVision FLEX Antibody Diluent). After incubation, slides were washed and incubated for 1 h in darkness with secondary antibodies (donkey monoclonal anti-mouse IgG with TRITC, 1:2,000 in EnVision FLEX Antibody Diluent). Samples were closed using Prolong with Dapi (Dako, Hamburg, Germany).

Statistical analysis. Statistical analyses were performed using STATISTICA (data analysis software system), version 10 (StatSoft, Inc., 2011) and PRISM7 (version number 7.02). In each cell population Kruskal-Wallis test was used to analyze the differences between three groups (meningioma, glioma, metastasis) in terms of the ratio of cells expressing a particular interleukin receptor to all cells in the population. Afterwards, three groups were compared pair-wise using Mann-Whitney test with Holm-Bonferroni correction for multiple comparisons. Wilcoxon signed-rank test was

performed to compare expression of two subunits of interleukin receptor (*i.e.* IL10Rα and IL10Rβ) in each cell population and group. The level of statistical significance was accepted as $p \leq 0.05$.

Results

An immunosuppressive phenotype of CD45$^+$ cells distinguishes primary tumors from metastases. The average percentage of CD45-positive cells in the given population equaled (mean±SD): 48,87±22,67% for meningiomas, 18,48±12,93% for gliomas and 20,30±19,21% for central nervous system metastases (Kruskal-Wallis test: $p=0.0086$). Mann-Whitney test with Holm-Bonferroni correction for multiple comparisons yielded the *p*-values as follows: $p=0.0096$ (meningiomas *vs.* gliomas), $p=0.03703$ (meningiomas *vs.* metastases) and $p=1.00$ (metastases *vs.* gliomas) (Figure 1A).

Properties of CD45$^+$ cells that differed significantly between the three groups were the mean expression of both subunits of IL10Rα ($p=0.015$) and β ($p=0.0074$). Mean ratio of CD45$^+$IL10Rα$^+$ cells to all CD45$^+$ cells was significantly higher in gliomas (53,03±15,87%) than in metastases (36,63±15,23%); $p=0.0356$. Moreover, it was also higher in meningiomas (55,22±12,96%) than metastases ($p=0.0365$) (Figure 1B). Significantly more CD45$^+$ cells expressed the β subunit of IL10R in gliomas (53,25±15,31%) than in metastases (36,64±15,91%); $p=0.0276$. Furthermore, its expression was higher in meningiomas (56,57±10,97%) than in metastases ($p=0.0235$; Figure 1C). The CD45$^+$IL10Rα$^+$/CD45$^+$ ratio did not differ significantly from the CD45$^+$ IL10Rβ$^+$/CD45$^+$ ratio in the three groups (meningiomas – $p=0.767$; gliomas – $p=0.754$; metastases – $p=0.790$), which may confirm that the two subunits occur together. In the assessment of the correlation between the two subunits, Spearman's rank correlation coefficient equaled 0.95, with S equal to 271.62 and p-value lower than 0.001 (Figure 1D).

Results obtained by flow cytometry are compatible with the evaluation of CD45$^+$ level by IHC and IF reactions. Immunochemical reaction was of a pronounced, membrane type. Both reactions were validated by comparative reactions on lymph nodes (Figure 2).

Discussion

Molecular differences between primary central nervous system tumors, such as glioblastoma, remain widely discussed when it comes to their classification, diagnostics criteria and potential new therapeutic approaches (23-24). Genomic instability of cancer cells is considered to be the primary source of their different molecular phenotypes (25). Apart from potential genetic biomarkers, protein markers are also of considerable significance in neurooncology.

Human Leukocyte Antigen (HLA) complex seems to be involved in the pathogenesis of some types of brain tumors, and immunological phenotype may have some importance in the regulation of carcinogenesis (26, 27).

Meningiomas show diverse expression of HLA-DR receptor even within the same histopathological phenotypes – early analyses of MHC II expression in different types of meningiomas show significant fluctuations in HLA-DR level, whereas its expression in the central nervous system is usually low and limited to microglia and perivascular macrophages (28). Among meningiomas there is also high heterogeneity of the immunophenotype of immunocompetent cells, especially of macrophages, NK cells and CD8$^+$ T lymphocytes (29).

The CD45 antigen, examined in this study is a marker of all leukocytes. It is regulating immunological response of immunocompetent cells, especially in T lymphocytes (30). In our study, we observed strong membrane immunohistochemical reaction of anti-CD45 antibodies in meningiomas, but also strong membrane-cytoplasmic reaction, which may suggest induction of expression of this protein by tumor cells.

Analysis of cells immunophenotype by flow cytometry is a sensitive and reliable method, useful in evaluation of tumor molecular characteristics (31). Our study investigated the correlation between the expression of IL-10 receptors in CD45$^+$ cells within brain neoplastic tissues. Flow cytometry analysis suggests that the population of CD45$^+$ cells expressing the IL-10 receptor may have some potential in distinguishing primary tumors and metastases. The immunophenotype of inflammatory infiltration cells differs significantly between tumor types. Both CD45$^+$IL10Rα$^+$/C45$^+$ and CD45$^+$IL10Rβ$^+$/CD45$^+$ ratios are lower in metastases than in both gliomas and meningiomas in this study. In all three tumor groups CD45$^+$IL10Rα$^+$/CD45$^+$ ratio correlated with CD45$^+$ IL10Rβ$^+$/CD45$^+$ ratio.

An interesting result of our study is the significantly higher level of CD45 expression in meningiomas than in glioblastomas and metastases. All metastases had epithelial genesis, were mostly adenocarcinomas, and gliomas have neuroepithelial genesis, in contrary to meningiomas, which are formed from arachnoid granulation.

Meninges represent physiological protection by isolating the nervous tissue from exposure to pathogens and other activation factors. In embryogenesis, they are formed from the mesenchyme and neural crest, whilst other brain elements are formed from neural tubes (32). These differences should be taken into consideration in future studies.

In a study by Stick *et al.*, there was no significant correlation between Leukocyte Common Antigen (LCA) and CD68 antigens in metastases and primary brain tumors, however their study was limited to immunohistochemical analysis and was performed on relatively few cases. However, the authors observed higher mean expression of

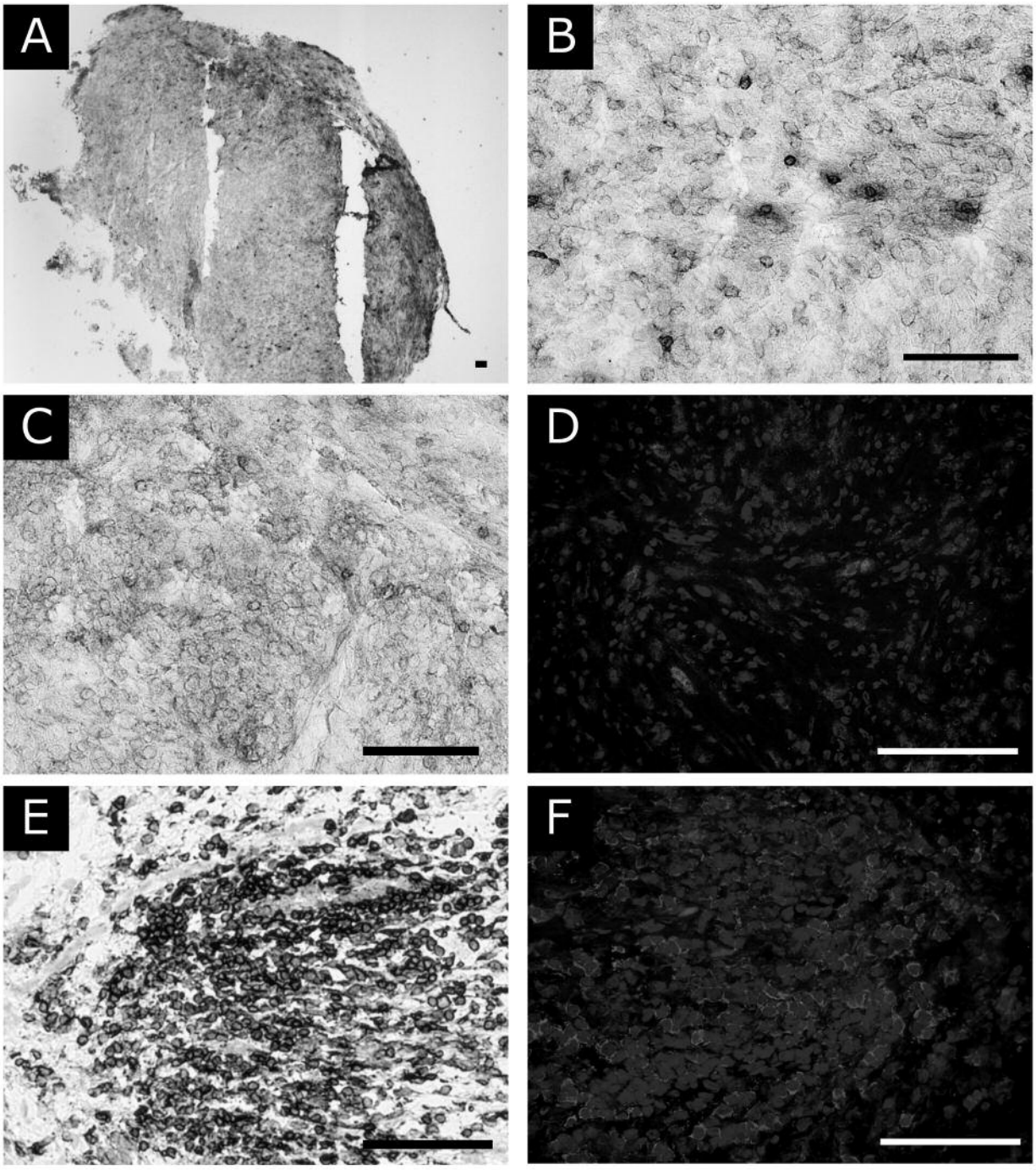

Figure 2. *Immunochemical (IHC and merged IF) analysis of CD45 expression. A-D: Meningioma. E-F: normal lymph node (positive control). D, F: Blue color – DAPI (cell nuclei), red – anti-CD45 reaction. Clear membrane type reaction is present. Scale bar=100 μm.*

the LCA antigen in meningiomas than in glioblastomas (33).

Our study has certain limitations. It was conducted on a relatively small and heterogenous population. Also, all the patients were treated with dexamethasone, as a glucocorticoid therapy against cerebral edema, it is a treatment of choice among brain tumor patients (34). Dexamethasone affects the immune system and can alter the profile of expressed cytokines, including IL-10 (35, 36), that can simultaneously disrupt the measurements of expression of investigated receptors. To eliminate the potential influence

of steroids on the obtained results, all the patients included in the study were administered the same dose of dexamethasone, equal to 8 mg daily. However, that limited the number of patients that could be included in the study group. Taking into consideration these issues and limitations, our results require further studies to provide more evidence and fully elucidate the influence of IL-10 receptor in central nervous system tumors.

Conclusion

$CD45^+$ cells are most numerous in meningiomas and both $CD45^+IL10R\alpha^+/CD45^+$ and $CD45^+IL10R\beta^+/CD45^+$ ratios are lower in metastases than in gliomas and meningiomas. This is a preliminary study suggesting the potential role of $IL10R^+/CD45^+$ as a differential marker between primary brain tumors and metastases. The role of IL10RA in brain tumors and its potential as a differential marker need further study and should be confirmed on larger population.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Acknowledgements

The study was funded by Poznań University of Medical Sciences and Department of Histology and Embryology in Wrocław.

The Authors would also like to express their gratitude to the Chair of Clinical Immunology at Poznań University of Medical Sciences for providing the flow cytometry reagents for investigation of both subunits of IL10 receptor.

References

1 Louis DN: Molecular pathology of malignant gliomas. Annu Rev Pathol *1*: 97-117, 2006.
2 Fathi AR and Roelcke U: Meningioma. Curr Neurol Neurosci Rep *13*: 337, 2013.
3 Gavrilovic IT and Posner JB: Brain metastases: epidemiology and pathophysiology. J Neurooncol *75*: 5-14, 2005.
4 Gajjar K, Heppenstall LD, Pang W, Ashton KM, Trevisan J, Patel II, Llabjani V, Stringfellow HF, Martin-Hirsch PL, Dawsonb T and Martin FL: Diagnostic segregation of human brain tumours using Fourier-transform infrared and/or Raman spectroscopy coupled with discriminant analysis. Anal Methods *5*: 89-102, 2012.
5 Tomita T, McLone DG and Naidich TP: Brain stem gliomas in childhood. Rational approach and treatment. J Neurooncol *2*: 117-122, 1984.
6 Weiss T, Weller M and Roth P: Immunological effects of chemotherapy and radiotherapy against brain tumors. Expert Rev Anticancer Ther *16*: 1087-1094, 2016.
7 Gajewski TF, Schreiber H and Fu YX: Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol *14*: 1014-1022, 2013.
8 Iwami K, Natsume A and Wakabayashi T: Cytokine networks in glioma. Neurosurg Rev *34*: 253-263; discussion 263-254, 2011.
9 Kumar R, Kamdar D, Madden L, Hills C, Crooks D, O'Brien D and Greenman J: Th1/Th2 cytokine imbalance in meningioma, anaplastic astrocytoma and glioblastoma multiforme patients. Oncol Rep *15*: 1513-1516, 2006.
10 Moore KW, de Waal Malefyt R, Coffman RL and O'Garra A: Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol *19*: 683-765, 2001.
11 Raphael I, Nalawade S, Eagar TN and Forsthuber TG: T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine *74*: 5-17, 2015.
12 Geginat J, Larghi P, Paroni M, Nizzoli G, Penatti A, Pagani M, Gagliani N, Meroni P, Abrignani S and Flavell RA: The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer. Cytokine Growth Factor Rev *30*: 87-93, 2016.
13 Ye Z, Huang H, Hao S, Xu S, Yu H, Van Den Hurk S and Xiang J: IL-10 has a distinct immunoregulatory effect on naive and active T cell subsets. J Interferon Cytokine Res *27*: 1031-1038, 2007.
14 Chan IH, Wu V, McCauley S, Grimm EA and Mumm JB: IL-10: Expanding the immune oncology horizon. Receptors Clin Investig *2*: 1-15, 2015.
15 Ruffell B, Chang-Strachan D, Chan V, Rosenbusch A, Ho CM, Pryer N, Daniel D, Hwang ES, Rugo HS and Coussens LM: Macrophage IL-10 blocks $CD8^+$ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. Cancer cell *26(5)*: 623-637, 2014.
16 Gousias K, Markou M, Arzoglou V, Voulgaris S, Vartholomatos G, Kostoula A, Voulgari P, Polyzoidis K and Kyritsis AP:. Frequent abnormalities of the immune system in gliomas and correlation with the WHO grading system of malignancy. J Neuroimmunol *226*: 136-142, 2010.
17 Zisakis A, Piperi C, Themistocleous MS, Korkolopoulou P, Boviatsis EI, Sakas DE, Patsouris E, Lea RW and Kalofoutis A: Comparative analysis of peripheral and localised cytokine secretion in glioblastoma patients. Cytokine *39*: 99-105, 2007.
18 Josephson K, Logsdon NJ and Walter MR: Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. Immunity *15*: 35-46, 2001.
19 Ding Y, Qin L, Zamarin D, Kotenko SV, Pestka S, Moore KW and Bromberg JS: Differential IL-10R1 expression plays a critical role in IL-10-mediated immune regulation. J Immunol *167*: 6884-6892, 2001.
20 Sabat R: IL-10 family of cytokines. Cytokine Growth Factor Rev *21*: 315-324, 2010.
21 Walter MR: The molecular basis of IL-10 function: from receptor structure to the onset of signaling. Curr Top Microbiol Immunol *380*: 191-212, 2014.
22 Wei SH, Ming-Lum A, Liu Y, Wallach D, Ong CJ, Chung SW, Moore KW and Mui AL: Proteasome-mediated proteolysis of the interleukin-10 receptor is important for signal downregulation. J Interferon Cytokine Res *26*: 281-290, 2006.
23 Forshew T, Lewis P, Waldman A, Peterson D, Glaser M, Brock C, Sheer D and Mulholland PJ: Three different brain tumours evolving from a common origin. Oncogenesis *2*: e41, 2013.
24 Habberstad AH, Lind-Landström T and Torp SH: The histopathological spectrum of primary human glioblastomas with relations to tumour biology. J Clinic Experiment Pathol *2*: 110, 2012.

25 Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, Esteller M, Fitzgerald R, Korbel JO, Lichter P, Mason CE, Navin N, Pe'er D, Polyak K, Roberts CW, Siu L, Snyder A, Stower H, Swanton C, Verhaak RG, Zenklusen JC, Zuber J and Zucman-Rossi J: Toward understanding and exploiting tumor heterogeneity. Nat Med *21*: 846-853, 2015.

26 Machulla HK, Steinborn F, Tschigrjai M, Langner J and Rainov NG: Meningioma: is there an association with human leukocyte antigens? Cancer Epidemiol Biomarkers Prev *12(12)*: 1438-1442, 2003.

27 Tang X, Wang S, An C, DU P and Yang Y: Preoperative high neutrophil-to-lymphocyte ratio is associated with high-grade bladder cancer. Anticancer Res *37(8)*: 4659-4663, 2017.

28 Ohara N, Hayashi K, Miyake K, Jeon HJ, Takahashi K and Akagi T: An immunohistochemical study on HLA-DR expression in human meningiomas. Acta Neuropathol *84(1)*: 110-112, 1992.

29 Domingues PH, Teodósio C, Ortiz J, Sousa P, Otero A, Maillo A, Bárcena P, García-Macias MC, Lopes MC, de Oliveira C, Orfao A and Tabernero MD: Immunophenotypic identification and characterization of tumor cells and infiltrating cell populations in meningiomas. Am J Pathol *181(5)*: 1749-1761, 2012.

30 Altin JG and Sloan EK: The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol *75(5)*: 430-445, 1997.

31 Rhone P, Ruszkowska-Ciastek B, Celmer M, Brkic A, Bielawski K, Boinska J, Zarychta E and Rosc D: Increased number of endothelial progenitors in peripheral blood as a possible early marker of tumour growth in post-menopausal breast cancer patients. J Physiol Pharmacol *68(Suppl 1)*: 139-148, 2017.

32 Beatriz M and Lopez S: Peritumoral edema. *In*: Meningiomas. Lee JH (eds.) Springer, London, 2009.

33 Strik HM, Stoll M and Meyermann R: Immune cell infiltration of intrinsic and metastatic intracranial tumours. Anticancer Res *24(1)*: 37-42, 2004.

34 Kaal EC and Vecht CJ: The management of brain edema in brain tumors. Curr Opin Oncol 16: 593-600, 2004.

35 Kakal JA, Ghazawi FM, Faller EM, Sugden SM, Parmar P and MacPherson PA: Transcriptional regulation of the IL-7Ralpha gene by dexamethasone and IL-7 in primary human CD8 T cells. Immunogenetics *69*: 13-27, 2017.

36 Hua F, Ji L, Zhan Y, Li F, Zou S, Wang X, Song D, Min Z, Gao S, Wu Y, Chen H and Cheng Y: Pulsed high-dose dexamethasone improves interleukin 10 secretion by $CD5^+$ B cells in patients with primary immune thrombocytopenia. J Clin Immunol *32*: 1233-1242, 2012.

Received July 18, 2017
Revised August 6, 2017
Accepted August 9, 2017