Immune Cell Infiltration of Intrinsic and Metastatic Intracranial Tumours

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Abstract. *Background: Immune escape is one prerequisite for the formation of neoplasms that is reflected by the pattern of immune cell infiltration. Abundant monocytic infiltration without apparent phagocytic activity is well known in human gliomas, while other types of human intracranial tumours have not yet been investigated. Materials and Methods: We analysed LCA-positive lymphocytes and CD68-positive macrophages/ microglia by immunohistochemistry in 67 intracranial neoplasms: 18 glioblastomas (GBM), 14 primitive neuroectodermal tumours and medulloblastomas (PNET), metastases of 9 adenocarcinomas and of 8 malignant melanomas, and 18 benign meningiomas. Results: Levels of monocytic infiltration in GBM and adenocarcinomas were higher than in PNET and meningiomas. Lymphocytes were rare in all tested tumours. No differences were found between all malignant neoplasms and benign meningiomas and between primary intracranial and metastatic tumours. Conclusion: Malignancy or primary intracranial origin seem not to be major determinants of immune cell infiltration. Different patterns of cytokine production may explain the differences in single tumour entities.*

Formation of benign or malignant tumours is a multistep process involving various prerequisites. Dedifferentiation of cells, deficient inhibition of proliferation, migration and invasion, or induction of tumour angiogenesis are active changes of cellular functions. On the other hand, newly forming tumours have to overcome the immune defense of the organism that may recognise and eliminate neoplastic cells (1).

Glioblastomas are neoplasms which efficiently escape the immune response (2). This seems to be one of several reasons for the aggressive behaviour of these tumours. In spite of substantial efforts during the last decades to improve

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antineoplastic therapy, glioblastomas are still associated with a median survival of only about 12 months (3).

Microglia are the tissue-specific antigen-presenting cells of the brain that are involved in virtually all pathologic processes in the brain (4). Morphologically, ramified microglia – which are considered to be resting – are distinguished from round microglia that are regarded as being active. In experimental and human gliomas, a low content of lymphocytes and abundant monocytic infiltration was described by several groups (5-10). Further differentiation of experimental gliomas by FACS-analysis showed that – in addition to resident microglia – also blood-derived macrophages infiltrate these tumours (11). There is no morphological evidence, however, for an efficient cellular anti-tumour response in human gliomas, indicating that infiltrating immune cells are inactivated before exerting immunologic functions (4).

In contrast to gliomas, little is known about immune cell infiltration and tumour-specific differences of immune cell reaction in other intracranial neoplasms. We therefore compared infiltration by monocytic and lymphocytic cells in intrinsic glioblastomas, PNET/ medulloblastomas and meningiomas with cerebral metastases of adenocarcinomas and malignant melanomas in order to gain information on the influence of cellular origin and malignancy and on the extent of immune cell infiltration.

Materials and Methods

Tissue samples. Paraffin-embedded tissue from 67 intracranial neoplasms was tested: 18 glioblastomas (GBM), 14 primitive neuroectodermal tumours and medulloblastomas (PNET), metastases of 9 adenocarcinomas and of 8 malignant melanomas, and 18 benign meningiomas WHO-grade I (Table I). All specimens were primary resections and obtained prior to radiotherapy or chemotherapy. The diagnoses were established according to the revised WHO classification (12).

Immunohistochemistry. Paraffin-embedded samples were immersed in 0.01 M citrate buffer and irradiated 5 x 5 min in a microwave oven. Endogenous peroxidase was blocked with 1% H_2O_2 in methanol, unspecific protein-binding domains with standard porcine serum (Seromed®, Biochrom, Berlin, FRG) for 15 min.

Table I. *Tumour-type, positivity to LCA or CD68 and percentage of ramified CD68-positive microglia.*

Number	Glioblastomas		
	LCA %	CD 68 %	ramified %
1	0.62	9.22	0.18
2	0.00	7.74	0.00
3	0.59	5.38	0.05
4	0.74	13.98	0.00
5	0.03	1.07	0.00
6	0.12	1.18	0.00
7	0.42	4.84	0.03
8	0.69	15.51	0.29
9	0.16	8.40	0.17
10	0.14	2.80	0.13
11	0.49	8.33	0.15
12	0.00	3.71	0.00
13	0.00	10.88	0.60
14	0.10	5.76	0.28
15	1.00	12.95	0.04
16	0.00	12.61	0.26
17	0.03	1.44	0.00
18	0.00	0.41	0.00
PNET/Medulloblastomas			
19	0.12	3.60	0.04
20	0.03	1.72	0.01
21	0.15	1.00	0.01
22	0.00	0.95	0.02
23	0.24	1.08	0.08
24	0.11	1.14	0.00
25	0.32	0.99	0.00
26	0.00	2.68	0.02
27	0.00	1.96	0.00
28	0.55	1.80	0.06
29	0.00	3.66	0.00
30	0.38	1.88	0.00
31	0.04	0.38	0.00
32	0.04	0.73	0.00
Adenocarcinomas			
33	10.20	12.68	1.59
34	1.53	14.51	0.20
35	0.33	2.42	0.00
36	0.75	2.66	0.00
37	0.09	4.92	0.15
38	0.00	5.87	0.15
39	1.78	5.51	0.08
40	0.33	4.10	0.00
41	0.78	2.55	0.00
Melanomas			
	LCA %	CD 68 %	ramified %
42	1.04	4.46	0.33
43	0.53	3.33	0.13
44	1.03	4.46	0.00
45	0.28	1.46	0.00
46	1.27	3.74	0.00
47	0.30	1.36	0.00
48	0.12	3.18	0.00
49	0.00	8.90	0.00

Table I (continued)

Meningiomas			
50	1.63	0.00	0.00
51	1.00	1.71	0.00
52	0.22	0.00	0.00
53	0.00	0.03	0.00
54	0.52	0.00	0.00
55	0.33	0.16	0.00
56	1.90	0.04	0.00
57	0.66	0.64	0.00
58	1.36	2.82	0.14
59	2.28	5.87	0.00
60	1.08	4.38	0.00
61	0.10	1.84	0.00
62	2.57	3.40	0.00
63	0.45	3.06	0.11
64	0.46	10.31	0.00
65	1.07	1.60	0.16
66	0.46	3.21	0.33
67	0.12	1.45	0.00

Monoclonal antibodies directed against LCA (DAKO, Glostrup, Denmark; 100 μl of a 1:100 dilution with blocking buffer: 0.025 M Tris-HCl pH 7.5, 0.1% BSA) or against the macrophage/ microglia marker CD68 (KP1, DAKO; 100μl at 1:150) were applied for 1 h. Sections were then incubated with biotinylated monoclonal anti-mouse IgG antibody fragment (DAKO) for 30 min, followed by streptavidin-biotin horseradish peroxidase complex (DAKO) diluted 1:200 for 30 min. Labelled antigens were made visible with diaminobenzidine (Fluka, Neu-Ulm, FRG) for 10 min. All sections were counterstained with hematoxylin.

Evaluation. Labelled cells in the tumour parenchyma were assessed in 4 high power fields as a percentage of the total number of cells. The infiltration zone, perivascular regions and the surrounding of tumour necroses were excluded. The percentage of labelled cells in each tumour entity was compared by the one-way ANOVA test. The results were controlled with the Bonferroni method for multiple significance testing. In order to gain information on the influence of the cellular origin or the grade of malignancy, we compared monocytic and lymphocytic infiltration in grouped primary intracranial GBM, PNET and meningiomas with metastatic tumours, and all malignant tumours with benign meningiomas with the one-way ANOVA test. CD68-positive cells were grouped as monocytes (microglia and macrophages) and evaluated morphologically for the presence of ramified microglia/ macrophages. The correlation with the total number of CD68-positive cells was analysed with the two-tailed Pearson test.

Results

LCA. The infiltration with cells positive to the leucocyte marker LCA was low in all tumours (Table II). With the exception of one adenocarcinoma, LCA-positive cells did not exceed 2% of all tumour cells. The distribution of these cells was irregular and not bound to morphologically distinct tumour regions. The median percentage was
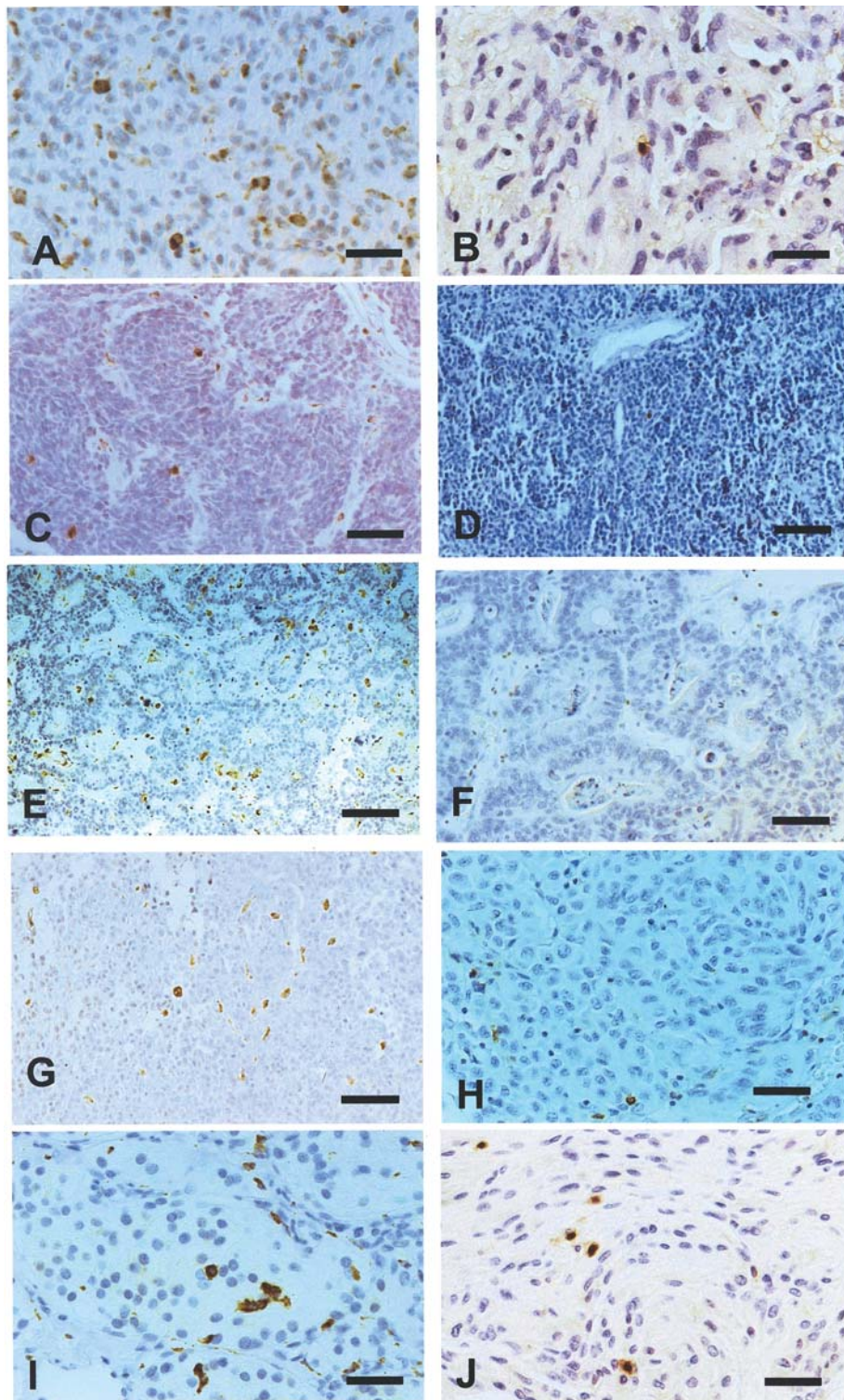
Figure 1. *Exemplary micrographs of positivity to CD68 (left column) and LCA (right column) in the tumour parenchyma. Positivity to CD68 was high in GBM (A, scale bar = 50 μm) and metastases of adenocarcinomas (E, scale bar = 25 μm), intermediate in metastases of malignant melanomas (G, scale bar = 25 μm) and low in meningiomas (I, scale bar = 50 μm) and PNET/ medulloblastomas (C, scale bar = 25 μm). Ramified microglia was rare in all examined tumours (not shown). Positivity to LCA was generally exhibited on a lower level. LCA-expression was higher in metastases of adenocarcinomas (F, scale bar = 50 μm), melanomas (H, scale bar = 25 μm) and in meningiomas (J, scale bar = 50 μm) than in GBM (B, scale bar = 25 μm) and PNET (D, scale bar = 50 μm).*

Table II. *Tumour-infiltration by immunocompetent cells. Median cumulative scores ± standard error of the mean (SEM) of the percentage of cells positive to immunohistochemical markers and p-values of the comparison of different tumour types by one-way ANOVA.*

	GBM n=18	PNET n=14	Adeno n=9	Melanoma n=8	Meningioma n=18	*p*
CD68	6.75 ± 1.13	1.43 ± 0.27	4.92 ± 1.48	3.54 ± 0.83	1.66 ± 0.62	GBM *vs.* PNET <0.0001 GBM *vs.* Meningioma <0.0001 PNET *vs.* Adeno <0.05
LCA	0.13 ± 0.07631	0.08 ± 0.04569	0.75 ± 1.075	0.42 ± 0.1695	0.59 ± 0.1821	PNET *vs.* Adeno 0.05
Ramified Microglia	0.045 ± 0.04	0.005 ± 0.01	0.080 ± 0.17	0.0 ± 0.04	0.0 ± 0.02	>0.05

highest in adenocarcinomas (1.08) and still higher in meningiomas (0.18) and melanomas (0.17) than in glioblastomas (0.08) and PNET/ medulloblastomas (0.05). Only the difference between the high percentage of LCA-positive cells in adenocarcinomas and the low rate in PNET/ medulloblastomas was significant.

CD68. The overall level of positivity to CD68 in all tumours was considerably higher than LCA-positivity. The distribution of CD68-positive cells was irregular, too, and could not be assigned to morphologically distinct areas. The median percentages were highest in glioblastomas and higher in adenocarcinomas or melanomas than in meningiomas or PNET/ medulloblastomas. The differences between glioblastomas and PNET/ medulloblastomas or meningiomas and between adenocarcinomas and PNET/ medulloblastomas were statistically significant.

The percentage of ramified microglia/ macrophages was below 1% in all tumours except the one adenocarcinoma with high infiltration by LCA- and CD68-positive cells. Considerable amounts of ramified microglia/ macrophages were detected in adenocarcinomas and glioblastomas and to a lesser extent in PNET/ medulloblastomas. Melanomas and meningiomas contained hardly any ramified microglia/ macrophages. The level of ramified cells correlated significantly with the total percentage of CD68-positive cells in glioblastomas and adenocarcinomas, but not in PNET/ medulloblastomas, melanomas and meningiomas.

General. It should be noted that positivity to CD68 was significantly higher in glioblastomas than in PNET and in meningiomas, while positivity to LCA in glioblastomas was at a level similar to that in PNET and tended to be lower than in meningiomas. By contrast, expression levels of both antigens were relatively high in adenocarcinomas and in melanomas and lower in meningiomas. No differences of positivity to LCA or CD68 was seen between benign meningiomas and pooled malignant tumours or between pooled primary intracranial GBM, PNET and meningiomas and pooled metastatic adenocarcinomas and melanomas (not shown).

Discussion

Previous studies on immune cell infiltration in human brain tumours focused on gliomas only (5,7,9,10). Our comparison showed no consistent differences between primary intracranial and metastatic CNS-tumours or between benign and malignant neoplasms.

The vast majority of monocytes was round and by such regarded as being activated (4). Phagocytosis, however, is not a typical feature in gliomas (4) and was not prominent in any other of the tumours examined here. Monocytic activation appears to have been blocked at some stage in all analysed tumours.

Surprisingly, no significant differences of monocytic infiltration were seen between pooled primary intracranial neoplasms and CNS metastases or between malignant tumours and benign menigiomas. The latter observation supports our previous study, where we found no significant correlation between WHO grade and the extent of monocytic infiltration in human gliomas (13).

In contrast to the abundant monocytic infiltration, the content of lymphocytes in glioblastomas was not significantly higher, but at a similar level to PNET/ medulloblastomas. Adenocarcinomas, however, contained a still considerable amount of lymphocytes, which was significantly higher than in PNET/ medulloblastomas. This finding is consistent with data from experimental primary and metastatic brain tumours (6) and indicates that the activation of lymphocytes by monocytes was deficient in all examined tumours, probably most strongly in glioblastomas.

In summary, the cellular origin or the grade of malignancy seem not to determine the extent of invasion of the examined tumours by immune cells. Also, the current knowledge on tumour-specific antigens can not explain the findings described here: no significant differences of immune cell infiltration were seen between gliomas, in which no tumour-specific antigen could be detected to date (4), and metastatic melanomas, which are known to exhibit several tumour-specific antigens (14).

Most probably, different expression levels of immune-modulatory cytokines in the tested tumours may explain the distinct patterns of immune cell infiltration. Glioma-cell-derived MCP1 (15) and HGF/ SF (16,17) can attract monocytes. In parallel, immunosuppressive IL-10 (18), TGF-β (2), or CD70 (19) apparently inactivate the immune response in gliomas. Thus, gliomas may both attract and inactivate monocytes. Moreover, the chemoattractant MCP-1 has been recently shown to increase not only microglial invasion, but also aggressiveness of experimental gliomas (20). This indicates that tumour-infiltrating monocytes may promote tumour growth. Levels of immunomodulatory cytokines in meningiomas, PNET/ medulloblastomas and metastases of systemic tumours have been poorly investigated to date. Comparative analyses of cytokines and chemokines in these tumours may contribute to a better understanding of immune reactions and to the development of immune therapies for intracranial tumours.

Acknowledgements

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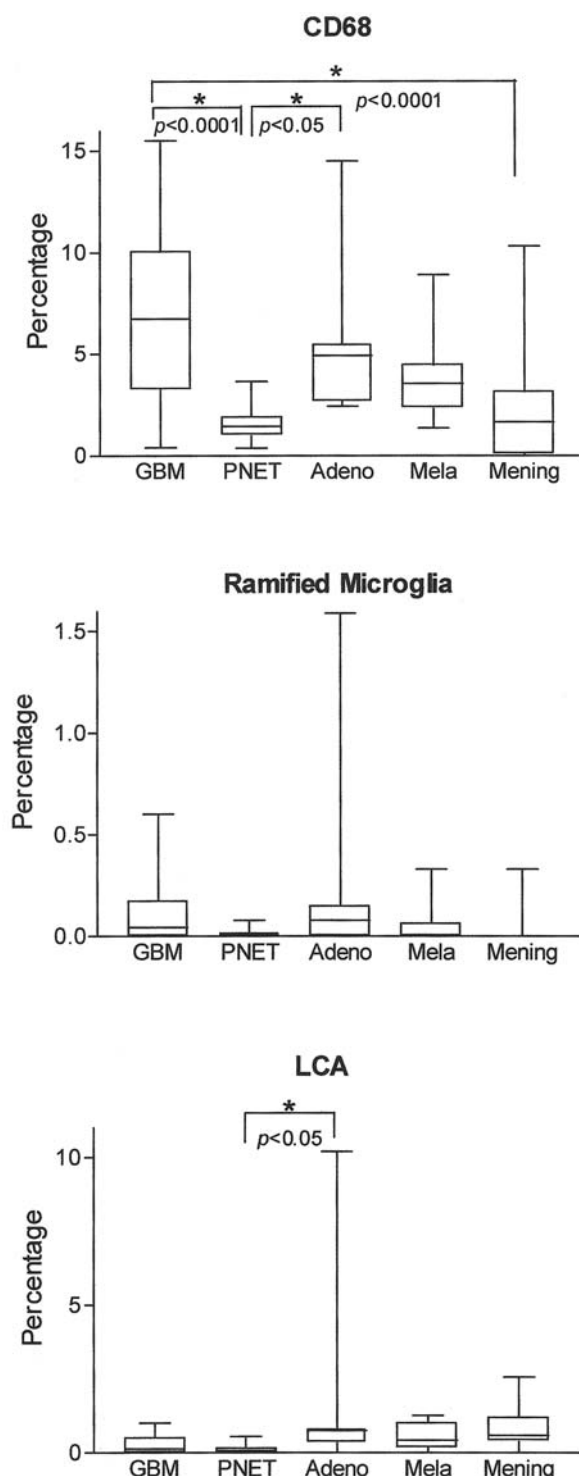

Figure 2. *Percentage of cells positive to CD68 or LCA and of microglial cells in the examined tumours. Center lines = median; boxes = quartiles (25 and 75% percentiles); whiskers = total range. The difference of cells positive to CD68 between GBM and PNET or meningiomas is significant. The difference between metastases of adenocarcinomas and PNET/ medulloblastomas is significant for both CD68 and LCA. Interestingly, in GBM, median positivity for CD68 is higher than in all other tumours, while expression of LCA is as low in GBM as in PNET.*

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