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Immunohistological investigation of mononuclear cell infiltrates in meningiomas

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Summary. Immunohistochemical analysis of inflammatory cell density and infiltrate subpopulations in 42 meningiomas was performed. Evaluation of infiltrating cell density was carried out by cell counting. Meningothelial and fibroblastic meningiomas contained an average of 3% mononuclear cells; the few lymphocytes were localized in the perivascular spaces. In subtypes with cellular atypies and recurrent tumors, the inflammatory cells increased up to 9%. We found small mononuclear cell clusters in the tumor parenchyma in addition to the perivascular infiltrates. Marked degrees of infiltration were found in anaplastic meningiomas (average 13.5% of total cells). The lymphocytic infiltrates were localized in multilayered perivascular cuffings and intraparenchymal cell clusters. The composition of the infiltrates, i.e., predominantly a mixed staining of cytotoxic/suppressor and helper cell phenotypes, did not vary in the different subtypes. We conclude: (1) that inflammatory infiltration is more frequent and denser in malignant than in benign meningiomas; and (2) that the tumor defense mechanisms in meningiomas are mediated particularly by T cell mediated immunity.

Key words: Meningioma – Mononuclear infiltration – Lymphocyte subset – Tumor immunology

Since the turn of the century, histopathological investigations have demonstrated leukocyte infiltration of tumors. Their prognostic significance, however, has not yet been fully clarified. Many authors described the occurrence of mononuclear infiltrates in brain tumors [3, 4, 6, 7, 16, 19, 23, 28, 32, 33, 36]; and most of them suggested that mononuclear cell infiltration had a defensive connotation and that lymphocytes were important in the host-mediated immunoreaction to neoplasm in the CNS.

A host-mediated immune response to brain tumors includes many interrelated humoral and cellular events that are affected by the subpopulations of mononuclear cells. Subtyping of these immunocompetent cells is necessary to clarify the role of lymphocyte subsets in this immunological reaction of the host.

After appropriate antibodies against mononuclear cells became available, several authors defined lymphocyte subsets in gliomas [9, 14, 18, 26, 29–31]. These immunohistochemical methods allowed definition of the subsets within the tumors, which, in turn, served to elucidate the role of the mononuclear cells in tumor surveillance.

To the best of our knowledge, only a few reports on inflammatory reactions in brain tumors other than gliomas have been published. Our study is based on the observation of infiltrating lymphocytes in meningiomas, primarily anaplastic meningiomas. This observation of large numbers of lymphocytes in anaplastic meningiomas and scattered lymphocytes in benign meningiomas, perhaps occasionally referred to as "pycnotic" cells, is still unclarified. These inflammatory infiltrates have not been analyzed extensively.

Our study examined 42 meningiomas using monoclonal antibodies against lymphocytic surface antigens to determine the density of infiltrating lymphocytes and the role of different immunocompetent cell subpopulations in the host-tumor interaction.

Methods

Over a 15-month period, unselected surgical biopsies of meningiomas for which snap-frozen specimens were available, were investigated for the presence of mononuclear infiltrating cells.

The various meningioma subtypes are presented in Table 2. Cryostat sections, 6 μm thick, from snap-frozen tumor specimens were prefixed for 10 min in acetone and for 30 min in

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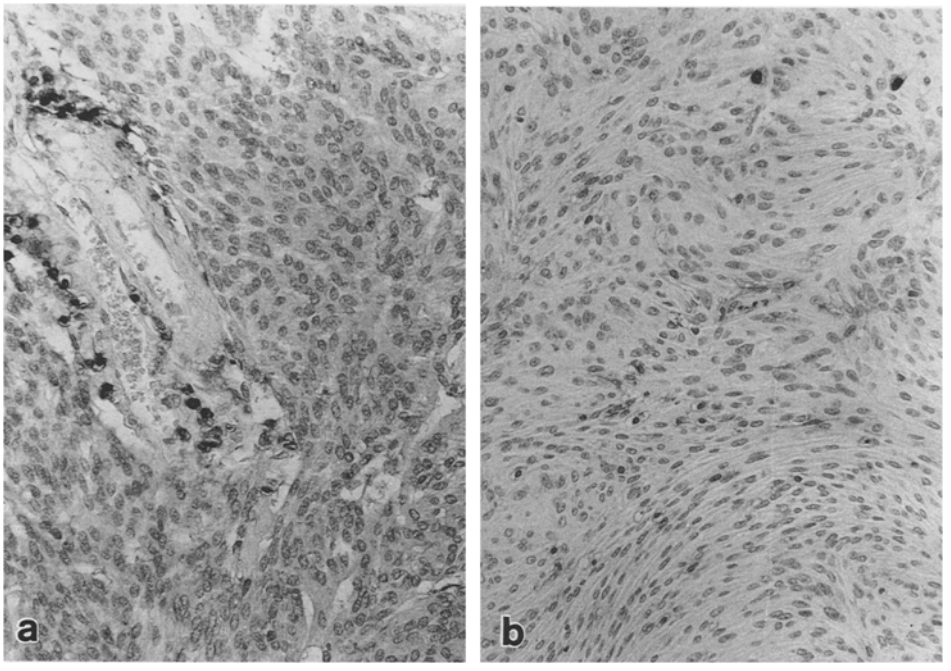

Figs. 1–3. Avidin-biotin-peroxidase complex staining method on paraffin sections with leukocyte common antigen

Fig. 1. In meningothelial and fibroblastic meningiomas few lymphocytic infiltrates were located in the perivascular interstitial tissue **(a)** or were scattered throughout the tumor **(b)**. **a, b** ×250

Table 1. Density of inflammatory cells in the meningioma subtypes, defined by a monoclonal antibody against the leukocyte common antigen (LC)

Histopathological diagnosis of the meningiomas	No. of cases	% LC-positive cells[a]	%Range
Meningothelial	11	3	1.9– 3.5
With atypies	4	8	5.1–10.7
Recurrent meningothelial	2	5	4.9– 5.2
Fibroblastic	5	3	2.0– 3.6
Fibroblastic with atypies	3	9	7.8–11.4
Recurrent fibroblastic	2	5.5	5.2– 5.9
Transitional-type	5	8.5	6.0–12.1
Anaplastic	5	13.5	8.4–16.9
Angioblastic	5	7.5	7.1–10.6

[a] The number of LC-positive cells is expressed as a percentage of the total cells present within a representative area of the tumor

chloroform at room temperature. The formalin-fixed, paraffin-embedded specimens were cut into 5-μm sections and dewaxed in xylene. The monoclonal antibodies used for incubations are given in Table 2. All antibodies were applied to cryostat sections and leukocyte common antigen (LC), human T cell, human B cell and Leu-7 antibodies were also applied to paraffin sections.

The avidin-biotin-complex reaction (ABC), as modified by Hsu et al. [12], and the alkaline phosphatase-antialkaline phosphatase method (APAAP), as modified by Cordell et al. [8], were used. As control for nonspecific staining the primary antibody was omitted. Frozen material from normal human rectal mucosa and submucosa served as positive controls.

The percentage of LC-positive cells was determined by counting 5000 to 6000 cells at a magnification of ×160 in a representative area of each biopsy. Comparison of the proportion of positive cells in the paraffin sections with that obtained in frozen sections showed the percentage to be identical. The specimens were examined for intratumoral, peritumoral, perivascular, and diffuse mononuclear infiltrates; localization and percentage of labelled cells were recorded.

Results

Meningothelial and fibroblastic meningiomas contain only scanty infiltrating lymphocytes. Specimens with increased cellularity or focal pleomorphism and the group of recurrent meningiomas showed a moderate number of infiltrating cells. The highest lymphocyte density was found in the anaplastic meningiomas (Table 1).

The few lymphocytic infiltrates in meningothelial and fibroblastic meningiomas were predominantly localized in the interstitial tissue surrounding tumor vessels (Fig. 1a). Only single LC-positive cells were found in areas with compact tumor formation or between the parallel and interlacing bundles of tumor cells (Fig. 1b). In the recurrent meningiomas and the meningiomas with atypical cells, scanty lymphocytic infiltrates were seen in the perivascular spaces. More-

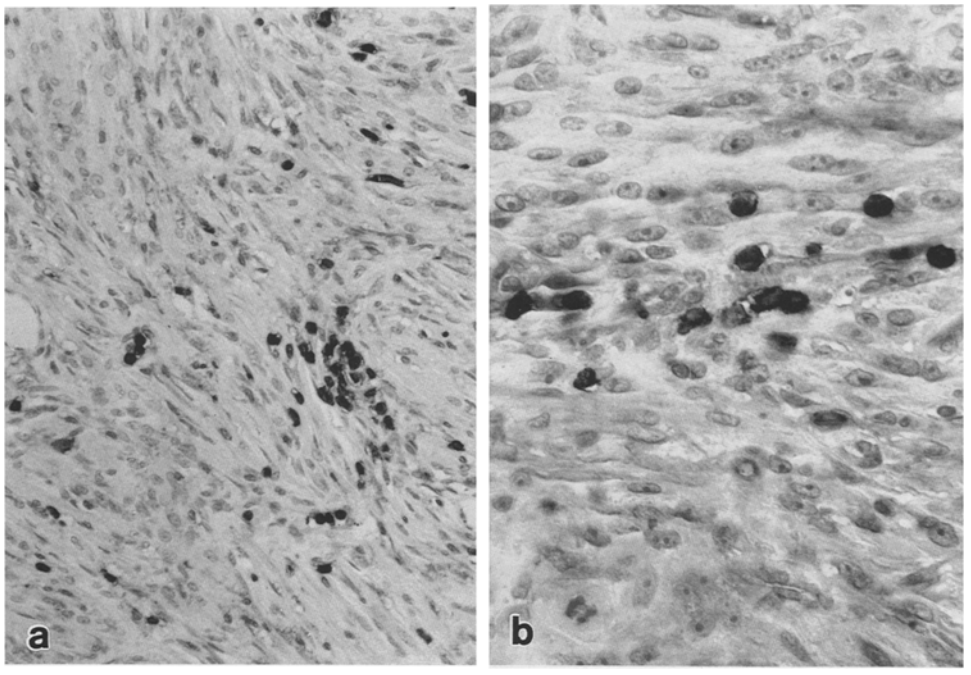

Fig. 2. **a** In meningiomas with atypic cells mononuclear cell clusters were seen in addition to scattered lymphocytes. **b** A small mononuclear cell cluster in a meningioma with cellular atypies and mitosis. **a** ×250, *b* ×410

Table 2. Monoclonal antibodies: subpopulations of leukocytes, which are particularly recognized by the mentioned antibodies and source

Antibody	Subpopulations
LC[a]	Leukocyte common antigen
Human T cell[a]	T lymphocytes (clone UCHL 1)
Human B cell[a]	B lymphocytes (clone 4KB5)
OKT8[b]	Cytotoxic/suppressor T lymphocytes
Leu-3a[c]	Helper/inducer T lymphocytes
Leu-M5[c]	Monocytes
Leu-7[c]	Natural killer cells
Leu-11b[c]	Natural killer cells
Leu-12[c]	B cells

[a] Dakopatts, Hamburg
[b] Ortho Diagnostics, Neckargemünd
[c] Becton Dickinson

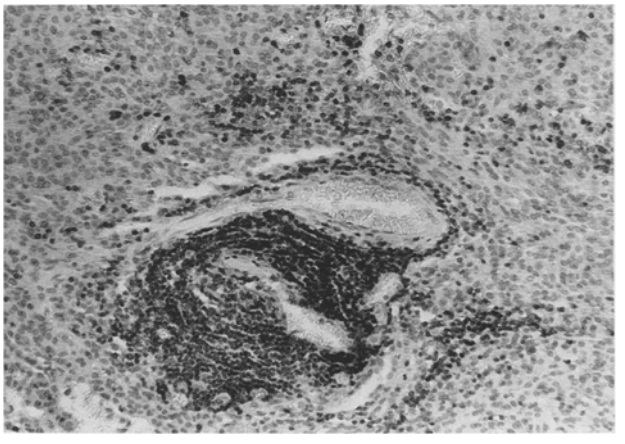

Fig. 3. An anaplastic meningioma with lymphocytes, forming perivascular cuffings, large and small intraparenchymal cell clusters. Single lymphocytes were frequent. ×160

over, we found small mononuclear cell clusters infiltrating the tumor parenchyma (Fig. 2a, b). The number of infiltrating lymphocytic cells was higher in transitional-type meningiomas than in meningothelial and fibroblastic meningiomas. The numerous lymphocytic infiltrates in anaplastic meningiomas consisted, for the most part, of multilayered perivascular cuffs as well as large and small intraparenchymal cell clusters; diffuse infiltration by small lymphocytes, however, was also a common finding (Fig. 3). The density of the infiltration varied in different areas of these tumors, increasing slightly in the border zone around areas of necrosis. Mononuclear cells were diffusely distributed in the angioblastic meningiomas.

The correlation between the density of infiltrating cells in the different histological meningioma subtypes and the proliferative potential of these tumors [24] is statistically significant, with the exception of angioblastic meningiomas, which have a relatively higher proportion of infiltrating cells, i.e., a lower prolifer-

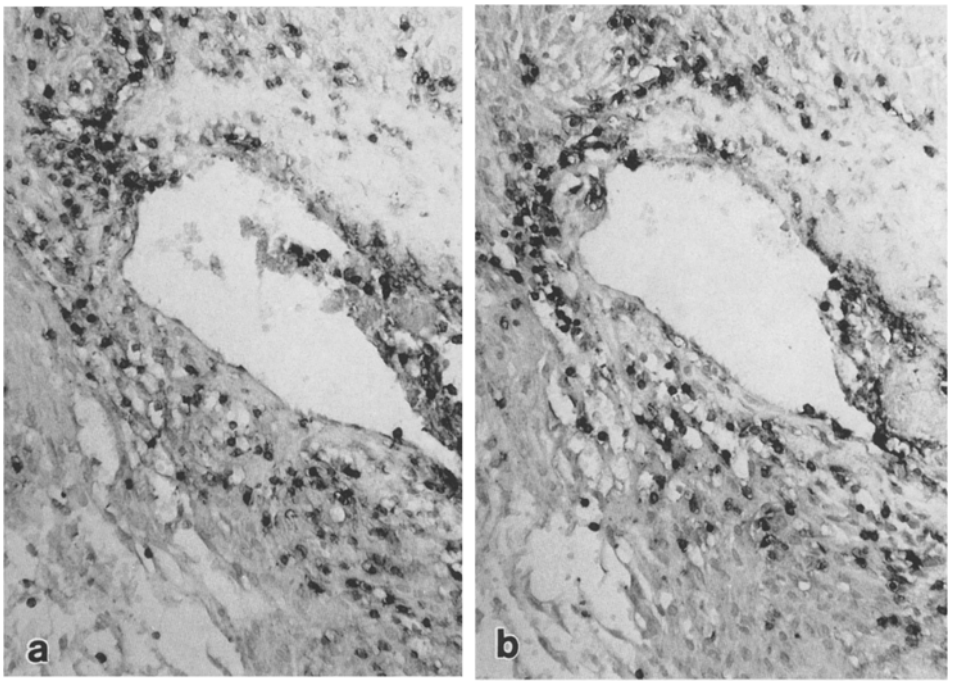

Fig. 4a, b. Serial frozen sections. **a** T helper lymphocytes (antibody Leu-3a); **b** suppressor-cytotoxic T lymphocytes (antibody OKT8). The infiltrates consisted mainly of T lymphocytes with a mixed T helper/cytotoxic population. Alkaline phosphatase antialkaline phosphatase, **a, b** $\times 250$

Table 3. Comparison of the density of inflammatory cells in the meningioma subtypes with the proliferation rate of these tumors

Histopathological diagnosis of the meningiomas	Number of cases	% of LC-positive cells[a]	Proliferation rate[b]
Meningothelial and fibroblastic	16	3.0	2.4
With cellular atypies and recurrent	11	8.5	8.6
Transitional-type	5	8.5	7.7
Anaplastic	5	13.5	14.1
Angioblastic	5	7.5	3.5

[a] The number of LC-positive cells is expressed as a percentage of the total cells present within a representative area of the tumor
[b] The percentage of proliferating cells (defined with the monoclonal antibody Ki 67 Dianova, Hamburg) was determined by counting 200 to 500 cells in a representative area of the tumor [24]

ation rate compared to the other subtypes (Table 3). For statistical analysis we used the *H*-test of Kruskal and Wallis and the *U*-test of Wilcoxon, Mann and Whitney.

The observed infiltrates consisted predominantly of T lymphocytes with a mixed helper/cytotoxic T cell population (Fig. 4a, b). Only a small proportion of B cells, monocytes and natural killer (NK) cells was present (15% or less of total infiltrating cell population). A specific pattern of the infiltrates, e.g., concentric distribution of cytotoxic T cells and T helper cells, was not detectable. The composition of the infiltrates did not vary in the different meningioma subtypes.

Discussion

Since lymphoid cellular infiltrates in brain tumors were described in the literature, the role of mononuclear cells in CNS tumors has been discussed and investigated [5, 7, 14, 21, 35–37]. All these experiments were performed almost exclusively on glial tumors. Only von Hanwehr [9] examined 14 surgical specimens of brain tumors, including 5 meningiomas, for the presence of T cells. We have determined the density of infiltrating lymphocytes and the different subpopulation of these mononuclear cells in 42 meningiomas.

In our study, the highest proportion of infiltrating cells was found in the anaplastic meningiomas, followed by meningiomas with cellular atypies. These observations are similar to those reported by Brooks et al. [6] and von Hanwehr [9] in gliomas. These authors demonstrated that inflammatory infiltration is more frequent and denser in malignant gliomas than in gliomas grade I–II WHO. von Hanwehr [9] found the

highest proportion of mononuclear infiltrating cells in anaplastic meningiomas and one fibroblastic meningioma.

In the literature [11, 13, 17, 27, 29, 34], one group of meningiomas is distinguished by the presence of very large numbers of lymphocytes, especially stimulated B lymphocytes. These cells may be present in such large numbers that they overshadow the underlying meningothelial islands. These lesions have been variously considered as true meningiomas, inflammatory masses with nests of trapped hyperplastic meningothelial cells, or aggregates of epithelioid histiocytes. No member of this special group of plasma cell-infiltrated tumor masses was present in our biopsies.

Our investigation of lymphocyte subsets showed that the tumor defense mechanism in meningiomas is mediated particularly by T cell-dependent immunity. NK cells seem not to play a major role in the immune response to meningiomas. The absolute numbers of NK cells can be still smaller in reality than the percentage of reacting cells with the antibody Leu-11 (CD16 antigen) and Leu-7 (HNK-1 glycoepitope), because some investigators could also demonstrate the CD16 antigen on cells of the mononuclear phagocyte system [1, 10] or the HNK-1 glycoepitope on various cell types including lymphocytic cells [1, 20, 22].

Other authors [9, 14, 31] defined T lymphocyte subsets in gliomas and found variable numbers of infiltrating T cells. Our previous study of infiltrate composition revealed only quantitative differences, the dominant cell population in gliomas, carcinoma metastases, and craniopharyngeomas, however, were T lymphocytes [31]. The usual evaluation of the helper/cytotoxic T-cell ratio should be considered with attention: the two major subsets of T cells identified by antibodies to CD4 (for example antibody Leu-3a) and CD8 (for example antibody OKT8) are commonly called the helper/inducer and suppressor/cytotoxic subsets. But this is a simplification because $CD4^+$ cytotoxic cells or $CD4^+$ monocytes and macrophages have frequently been detected. Therefore, the CD4/CD8 ratio can be falsified and this can perhaps explain the contradictory results in the various investigations [2, 9, 22, 31]. Mork et al. [18] found the predominant cell population in a small series of gliomas to be macrophages. Rossi et al. [26] described both macrophages and T lymphocytes in malignant gliomas. The determination of macrophages is problematical, because although the peripheral blood monocytes are a relatively homogeneous population, tissue macrophages and related dendritic cells are extremely heterogeneous in phenotype [2, 15, 22]. Thus, the most useful markers are probably those which identify all monocytes/macrophages and distinguish them from other cell types, but such a marker does not exist to the best of our knowledge. The available antibodies recognize peripheral blood monocytes or different stages of development of macrophages. Therefore, the recognition of the percentage of the actual macrophages in a tumor is dependent on the used antibody.

We presume that several factors are responsible for the variations we observed in the intensity of the immunological defense mechanism, such as: a variously pronounced alteration of antigenic determinants on the surface of tumor cells; the occurrence of neoantigens in the meningioma subtypes; and a possibly variable expression of major histocompatibility complex (MHC) antigens of the tumor and endothelial cells, which are necessary for an activation of T lymphocytes [25]. Analysis of MHC expression of tumor cells should provide new insights into the defense mechanisms of brain tumors.

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References

1. Baumgartner I, Scheiner O, Holzinger C, Boltz-Nitulescu G, Klech H, Lassmann H, Rumpold H, Förster O, Kraft D (1988) Expression of the VEP13 Antigen (CD16) on native human alveolar macrophages and cultured blood monocytes. Immunobiology 177:317–326
2. Bernard A, Bournsell L, Dausset J, Milstein C, Schlossman SF (1984) Leukocyte typing. Proc 1st Intern Workshop Leukocyte Differentiation Antigens. Springer, Berlin Heidelberg New York, pp 148–157
3. Bertrand I, Mannen H (1960) Études des réactions vasculairés dans les astrocytomes. Rev Neurol (Paris) 102:3–19
4. Böker DK, Kalff R, Gulotta F (1984) Mononuclear infiltrates in human intracranial tumors as a prognostic factor. Influence of preoperative steroid treatment. Clin Neuropathol 3:143–147
5. Brooks WH, Netsky MG, Normansell DE (1972) Depressed cell-mediated immunity in patients with primary intracranial tumors. J Exp Med 136:1631–1647
6. Brooks WH, Caldwell HD, Mortara RH (1974) Immune response in patients with gliomas. Surg Neurol 2:419–423
7. Brooks WH, Markesbery WR, Gupta GD (1978) Relationship of lymphocyte invasion and survival of brain and tumor patients. Ann Neurol 4:219–224
8. Cordell JL, Falini B, Erber WN (1984) Immunoenzymatic labelling of monoclonal anti-alkaline phosphatase. J Histochem Cytochem 32:319–329
9. Hanwehr RI von, Hofman FM, Taylor CR (1984) Mononuclear lymphoid populations infiltrating the microenvironment of primary CNS tumours. J Neurosurg 60:1138–1147
10. Hoog N, Horton MA (1987) Myeloid antigens: new and previously defined clusters. In: McMichael AJ (ed) Leukocyte typing III. Oxford University Press, Oxford, p 576
11. Horten BC, Urich H, Stefoski D (1979) Meningiomas with conspicuous plasma cell-lymphocytic components. A report of five cases. Cancer 43:258–364

12. Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580
13. Kepes JJ (1982) Meningiomas. Biology, pathology, and differential diagnosis. In: Kepes JJ (ed) Masson monographs in diagnostic pathology. Masson, New York, pp 111–112
14. Kril MP, Apuzzo LJ (1983) Observations in the study of T lymphocyte subsets by monoclonal antibodies and flow cytometric analysis in intracranial neoplastic disorders. Clin Neurosurg 30:125–136
15. McMichael LJ, Fabre J (1982) Monoclonal antibodies in clinical medicine. Academic Press, London, pp 62–75
16. Meyer-Rienecker HJ, Jenssen HL, Mix E (1985) Heterogene Immunreaktivität bei Hirntumoren: Ergebnisse zur zellulären Immunität gegenüber Hirngewebsantigenen. Fortschr Neurol Psychiatr 53:363–369
17. Mirra SS, Tindall SC, Check IJ, Brynes RK, Moore WW (1983) Inflammatory meningeal masses of unexplained origin. An ultrastructural and immunological study. J Neuropathol Exp Neurol 42:453–468
18. Mork SJ, Nyland H, Matre R, Ganz J (1985) Characterization of host mononuclear cells in gliomas. J Neuropathol Exp Neurol 44:317 [abstr]
19. Palma L, Di Lorenzo N, Guidetti B (1978) Lymphocytic infiltrates in primary glioblastomas and recidivous gliomas. J Neurosurg 49:854–861
20. Perentes E, Rubinstein LJ (1986) Immunohistochemical recognition of human neuroepithelial tumors by Anti-Leu-7 (HNK-1) monoclonal antibody. Acta Neuropathol (Berl) 69:227–233
21. Phillips JP, Eremin O, Anderson JR (1982) Lymphoreticular cells in human brain tumours and in normal brain. Br J Cancer 45:61–69
22. Reinherz EL, Haynes B, Nadler L, Bernstein I (1986) Leukocyte typing II. Proc 2nd Intern Workshop Leukocyte Differentiation Antigens. Springer, Berlin Heidelberg New York, pp 189–197
23. Ridley A, Cavanagh JB (1971) Lymphocytic infiltration in gliomas: evidence of possible host resistance. Brain 94: 117–124
24. Roggendorf W, Schuster T, Peiffer J (1987) Proliferative potential of meningiomas determined with the monoclonal antibody Ki 67. Acta Neuropathol (Berl) 73:361–364
25. Roitt IM, Brostoff J, Male DK (1985) Immunology. Gower Medical Publishing, London New York, pp 4.8–4.11
26. Rossi ML, Hughes JT, Esiri MM, Coakham HB, Brownell DB (1987) Immunohistological study of mononuclear cell infiltrate in malignant gliomas. Acta Neuropathol (Berl) 74:269–277
27. Russell DS, Rubinstein LJ (1977) Pathology of tumours of the nervous system. Williams & Wilkins, Baltimore, pp 330–335
28. Schiffer D, Croveri G, Pautasso C (1974) Frequenza e significato degli infiltrati linfo-plasmacellulari nei gliomi umani. Tumori 60:177–184
29. Stam FC, van Alphen HAM, Boorsma DM (1980) Meningioma with conspicuous plasma cell component. A histopathological and immunohistochemical study. Acta Neuropathol (Berl) 49:241–243
30. Stavrou D, Anzil AP, Weidenbach W, Rodt H (1977) Immunofluorescence study of lymphocytic infiltration in gliomas. Identification of T lymphocytes. J Neurol Sci 33:275–282
31. Stevens A, Klöter I, Roggendorf W (1988) Inflammatory infiltrates and NK cells presence in human brain tumors. Cancer 61:738–743
32. Takakura K, Sing OK (1972) Inhibitory role of immunologically competent cells on glioblastoma and its clinical application: in vitro studies. In: Fusek J, Kunc Z (eds) Present limits of neurosurgery. Excerpta Medica, Amsterdam, pp 47–51
33. Takeuchi J, Barnard RO (1976) Perivascular lymphocytic cuffing in astrocytomas. Acta Neuropathol (Berl) 35:265–271
34. West SG, Pittman DL, Coggin JT (1980) Intracranial plasma cell granuloma. Cancer 46:330–335
35. Wood GHW, Morantz RA (1979) Immunohistologic evaluation of the lymphoreticular infiltrate of human central nervous system tumours. J Natl Canc Inst 62:485–491
36. Wood GW, Morantz RA, Tilzer SA (1980) Immunoglobulin bound in vivo to Fc receptor-positive cells in human central nervous system tumours. J Natl Canc Inst 64:411–418
37. Young HF, Sakales R, Kaplan AM (1976) Inhibition of cell-mediated immunity in patients with primary intracranial tumors. Surg Neurol 5:19–23

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