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The mononuclear cell infiltrate compared with survival in high-grade astrocytomas*

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Summary. Frozen samples from 92 malignant astrocytomas were stained with a panel of monoclonal antibodies directed against macrophages and lymphocytes. A follow-up to death was available on 68 cases which form the basis of this study. Large numbers of macrophages were found in all cases; T lymphocytes, mostly of the CD8 phenotype were also seen in moderate numbers in 70% of cases. CD4-positive cells were present in small numbers in 32% and B cells were seen in only 8% of cases. Analysis of the survival showed no demonstrable correlation between the numbers of macrophages or CD4 lymphocytes and survival. The survival curves for parenchymal CD8 infiltration diverged after 9 months suggesting increased survival for those patients without such an infiltration but the difference failed to reach statistical significance ($P = 0.37$). No correlation between lymphocytic cuffing and survival was seen after studying all paraffin-embedded material. We conclude that there is no significant statistical correlation between survival and the various types of mononuclear cell infiltrating malignant astrocytomas.

Key words: Astrocytoma – Macrophage – Lymphocyte – Mononuclear cells – Survival

For some time it has been thought that mononuclear cells may be part of the host defence mechanism against tumours [1, 2, 36]. The lymphocytic infiltrate in human gliomas has been the subject of several studies [4, 26, 30], including some which suggest a favourable effect on survival [6, 24]. More recent studies have used monoclonal antibodies in attempts to characterize the subsets of infiltrating mononuclear cells [15, 18, 22, 23, 27–29, 31, 35].

Recently we reported a large study using a panel of monoclonal antibodies and concluded that the mononuclear infiltrate in high-grade astrocytomas is predominantly composed of macrophages with a smaller component of CD8 lymphocytes [27]. We now report additional data on the survival of some of those patients and some others which have since become part of a comprehensive follow up study of various CNS tumors.

Material and methods

Paraffin-embedded and snap-frozen material from 92 cases of astrocytoma Kernohan grades 3 and 4 [17] was obtained at operation. At the time of writing, follow-up was available on 68 of these patients, all of whom had died, and our analysis is limited to this group. The study group was compared with the group without follow-up data using chi-square analysis and no significant differences were found for any of the variables analysed in this report.

Cryostat sections were cut from the frozen blocks and stained using the indirect immunoperoxidase technique as before [27] with a panel of antibodies against lymphocyte subsets (including CD8 or T8 cytotoxic/suppressor and CD4 or T4 helper/inducer lymphocytes) and macrophages (Table 1).

The density of positive cells in parenchymal and perivascular areas, but not in necrotic areas or at their edges, was assessed using a scale from 0–4, where 0 indicated no positive cells; 1: occasional positive cells/high-power field (hpf) with a ×40 objective; 2: up to 20 cells/hpf; 3: from 20–40 cells/hpf, and 4: more than 40 cells/hpf. Perivascular lymphocytic cuffing on haematoxylin and eosin sections was graded as absent, mild, moderate or heavy.

The comparison of the variables studied with survival from the time of biopsy was assessed on a Vax (Digital Equipment Corporation) computer using the SPSSx (SPSS Inc.) program. Life table analysis was used and significance was assessed with the algorithm of Lee and Desu [19]. Because there were so few cases completely devoid of macrophages, sections graded as 0 or 1 (occasional cells/hpf) were called negative and all others positive for this group.

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Results

Results are illustrated in Table 2 and Fig. 1–5. Examples of the reactions obtained with this panel of monoclonal antibodies have already been illustrated [27].

DC8 lymphocyte

The number of cells detected by the antibody cocktail to T lymphocytes and the T8 antibodies were similar. CD8-positive cells were present in 56/68 (82%) of cases and when the parenchymal and perivascular locations were considered separately, they were seen in 48/68 (70%, mean cellularity 0.71) in the former and 41/64 (64%, mean cellularity 0.76) in the latter.

The survival curves for CD8 (Fig. 1) diverged after 9 months suggesting increased survival for those patients without such infiltration. When the site of infiltration (perivascular or parenchymal) was compared, the difference was seen to be almost entirely due to the parenchymal group (Fig. 2). Despite this appearance, the difference failed to reach statistical significance with a P value of 0.35 for the group overall.

Table 1. Summary of monoclonal antibodies and their dilutions used in this study

Monoclonal antibody [dilution]	Isotypes	Specificity	Source/ reference
Y182A [1:1]	IgG3	Macrophages	K. Gatter, Oxford [11]
RFBCT [1:1]	Cocktail IgM(CD20) IgG(CD20) RFBT	B lymphocytes	G. Janossy
RFTCT [1:1]	Cocktail IgG(CD2) IgG(CD3) IgG(CD7) IgG(CD8Y)	T lymphocytes	G. Janossy
T8 [1:10]	IgG1	CD8 lymphocytes	Dako
T4 [1:10]	IgG1K	CD4 lymphocytes	Dako

CD4 lymphocytes

CD4-positive cells were present in small numbers in parenchyma in 22/68 cases (32%, mean cellularity 0.31). There was no correlation with survival ($P = 0.67$) (Fig. 3).

Macrophages

Macrophages were found in large numbers in the parenchyma in all 68 assessable cases (Mean cellularity 2.5) and in perivascular spaces in 58/63 (92%, mean cellularity 1.9). Life table analysis was performed comparing all grades of infiltration with survival for both perivasular and parenchymal sites and, because of the relatively small number of completely negative cases, calculations were repeated after combining grades together. No statistically significant correlation was found for any combination. Comparing grades 0–1 with grades 2–4 for perivascular and parenchymal sites combined resulted in a P value of 0.39. Similar results were obtained when the site of infiltration was controlled for (perivascular $P = 0.50$, parenchymal $P = 0.34$) (Fig. 4a, b).

Lymphocytic cuffing

Perivascular lymphocytic cuffing in haematoxylin and eosin sections was found to be heavy in 3 cases, moderate in 2, mild in 27 and absent in 34. No assessement was possible in 4 cases due to insufficient material. Figure 5 shows the survival curves when cases with infiltrate were compared to those with none. No statistically significant difference ($P = 0.93$) was found.

Table 2. Summary of semiquantitative observations of macrophage and lymphocyte numbers

Semiquantitative rating	M par	M pv	CD8 par	CD9 pv	CD4
0	–	5	20	23	46
1	7	14	44	34	22
2	23	27	4	6	–
3	30	15	–	1	–
4	8	2	–	–	–
Total	68	63	68	64	68
% Positive	100	92	70	64	32

M, Macrophage; par, parenchymal; pv, perivascular

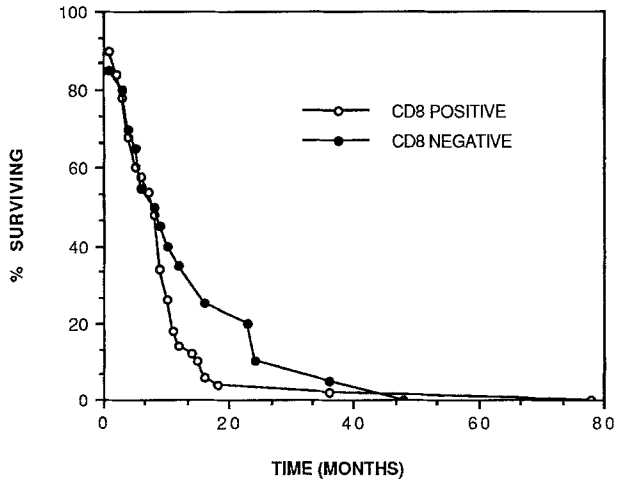


Fig 1. Survival curves for CD8 lymphocyte infiltration (perivascular and parenchymal). Grade 0: negative; grades 1–4: positive

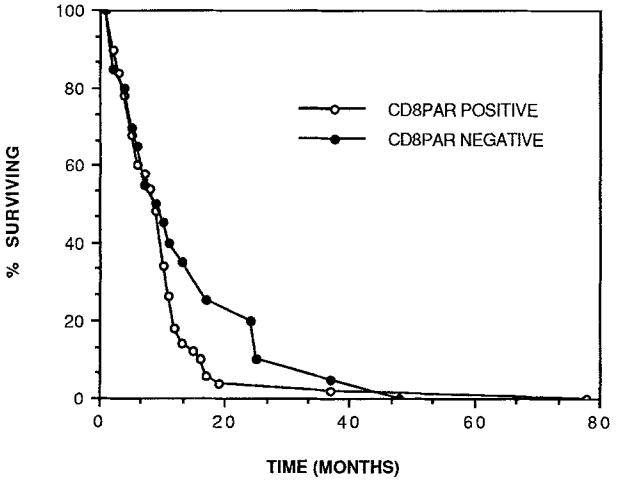


Fig 2. Survival curves for CD8 parenchymal lymphocyte infiltration. Grade 0: negative; grades 1–4: positive

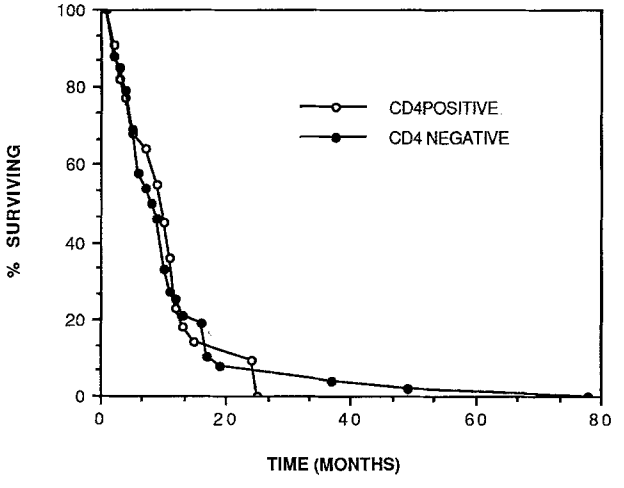
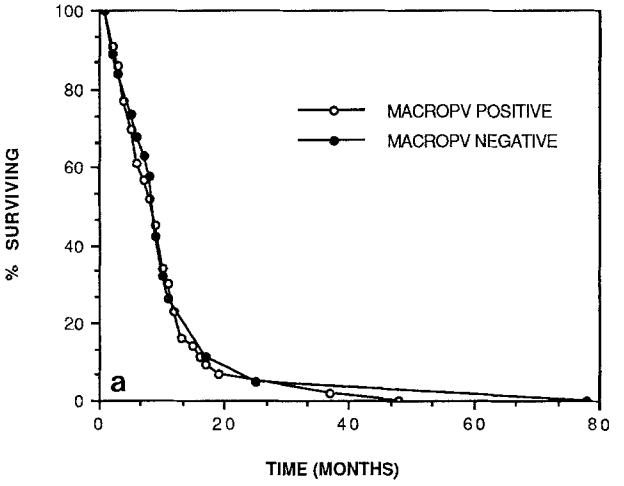


Fig 3. Survival curves for CD4 lymphocyte infiltration. Grade 0: negative; grades 1–4: positive



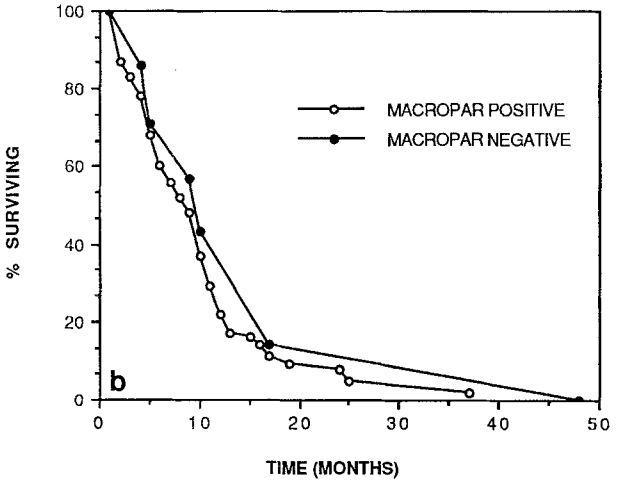


Fig 4. a Survival curves for perivascular macrophage infiltration. **b** Survival curves for parenchymal macrophage infiltration. **a, b** Grades 0–1: negative; grades 2–4: positive

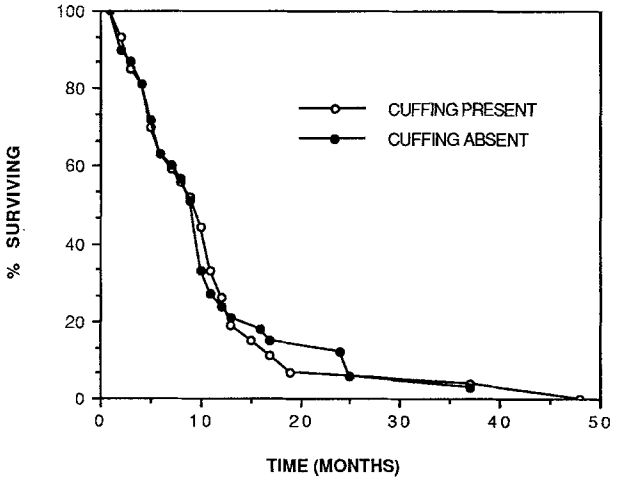


Fig 5. Survival curves for perivascular lymphocytic cuffing on haematoxylin and eosin sections

Discussion

During the past 30 years several reports have addressed the significance of mononuclear cells in intrinsic and extrinsic CNS tumours, both within the tumour itself [3, 4, 22, 24, 26, 30, 31, 33, 38] and in the peripheral blood [5, 13, 21, 25].

Improved survival has been correlated with a heavier lymphocytic infiltrate in paraffin sections [6, 24] but statistical analysis of our results indicates that no prognostic significance can be attributed to the presence of infiltrating lymphocytes, either in haematoxylin and eosin sections or as detected by immunocytochemistry.

Patients with malignant gliomas have a variety of immunological deficits including delayed hypersensitivity to recall antigens, decreased percentage of circulating T lymphocytes and impaired mitogen-induced blastogenic response by peripheral mononuclear cells (reviewed by Bullard [7, 8]). Deficits may result from an abnormality of T cell function and in particular of the CD4 lymphocytes and of interleukin 2-mediated effects [14]. Such impairment would lead to a decreased cell-mediated immune response including failure to generate sufficient numbers of cytotoxic T cells, whilst the macrophage-driven stimulation of lymphocytes by means of interleukin 1 could still remain normal [12]. If this is the case, no difference in host resistance to gliomas with varying degrees of CD4 and CD8 infiltrates may be expected whilst the number of macrophages could still be very large and not influence the prognosis. This in turn may provide an explanation, albeit simplistic, for our findings.

Despite our lack of correlation between the degree of macrophage infiltrate and survival within this group of malignant gliomas, we have previously observed a significant difference in the numbers of infiltrating macrophages in high- [27] and low-grade astrocytomas [28] and oligodendrogliomas [29], whilst no significant difference was observed for T or B lymphocytes. The larger number of macrophages found in high-grade gliomas could result from increased cell degeneration although we purposely avoided assessing frankly necrotic areas or their environs. On the other hand it may be that there is a relationship between the number of macrophages and glial tumour growth as these cells can not only induce (by means of tumour necrosis factor production) tumour cell death in tissue culture and involution of tumours in animals [9, 37] but also stimulate the growth of a variety of cells [10, 16, 20, 32, 34, 38].

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References

1. Alexander P (1967) Cellular resistance to tumours. Br Med Bull 23:86–92
2. Alexander P (1976) The functions of the macrophages in malignant disease. Annu Rev Med 27:207–224
3. Apuzzo MLJ, Mitchell MS (1981) Immunological aspects of intrinsic glial tumours. J Neurosurg 55:1–18
4. Bertrand I, Mannen H (1960) Etudes des reactions vasculaires dans les astrocytomes. Rev Neurol (Paris) 102:3–19
5. Brooks WH, Roszman TL, Rogers AS (1976) Impairment of rosette-forming T lymphocytes in patients with primary intracranial tumours. Cancer 37:1869–1873
6. Brooks WH, Markesbery WR, Gupta GD, Roszman TL (1978) Relationship of lymphocyte invasion and survival of brain tumour patients. Ann Neurol 4:219–224
7. Bullard DE, Bigner DD (1985) Applications of monoclonal antibodies in the diagnosis and treatment of primary brain tumours. J Neurosurg 63:2–16
8. Bullard DE, Gillespie GY, Mahaley MS, Bigner D (1986) Immunobiology of human gliomas. Semin Oncol 13:94–104
9. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumours. Proc Natl Acad Sci USA 72:3666–3671
10. Cordingley FT, Hoffbrand AV, Heslop HE, Turner M, Bianchi A, Reittie JE, Vyakarnam A, Meager A (1988) Tumour necrosis factor as an autocrine tumour growth factor for chronic B cell malignancies. Lancet I:969–971
11. Davey SR, Cordell JR, Erber WN, Pulford KAI, Gatter KC, Mason DY (1988) A monoclonal antibody (Y182A) with specificity towards peripheral blood monocytes and tissue macrophages. J Clin Pathol 41:753–758
12. Elliott LH, Brooks WH, Roszman TL (1984) Cytokinetic basis for the impaired activation of lymphocytes from patients with primary intracranial tumours. J Immunol 132:1208–1215
13. Elliott LH, Brooks WH, Roszman TL (1987) Activation of immunoregulatory lymphocytes obtained from patients with malignant gliomas. J Neurosurg 67:231–236
14. Fontana A, Hengartner H, de Tribolet N, Weber E (1984) Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated effects. J Immunol 132:1837–1844
15. Hitchcock ER, Morris CS (1988) Mononuclear cell infiltration in central portions of human astrocytomas. J Neurosurg 68:432–437
16. Kehrl JH, Miller A, Fauci AS (1987) Effects of TNF alpha on mitogen-activated human B cells. J Exp Med 166:786–791
17. Kernohan JW, Mabon RF, Stein JH, Adson AW (1949) A simplified classification of the gliomas. Proc Staff Meet Mayo Clin 24:71–75
18. Kril MP, Apuzzo MLJ (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
19. Lee E, Desu M (1972) A computer program for comparing k samples with right-censored data. Comput Programs Biomed 2:315–321

20. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N (1987) Macrophage-induced angiogenesis mediated by tumour necrosis factor-alpha. Nature 329:630–632
21. Mahaley MS, Brooks WH, Roszman TL, Bigner DD, Dudka L, Richardson S (1977) Immunobiology of primary intracranial tumours. Part 1: Studies of the cellular and humoral general immune competence of brain tumour patients. J Neurosurg 46:467–476
22. Morantz RA, Wood GW, Foster M, Clark M, Gollahon K (1979) Macrophages in experimental and human tumours. Part 2: Studies of the macrophage content of human brain tumours. J Neurosurg 50:305–311
23. Mork SJ, Nyland H, Matre R, Ganz J (1985) Characterization of host mononuclear cells in gliomas. J Neuropathol Exp Neurol [Abstr] 44:317
24. Palma L, Di Lorenzo N, Guidetti B (1978) Lymphocytic infiltrates in primary glioblastomas and recidivous gliomas: incidence, fate and relevance to prognosis in 228 operated cases. J Neurosurg 49:854–861
25. Rainbird S, Alwood G, Ridley A (1981) Lymphocyte-mediated cytotoxicity against gliomas. Brain 104:451–464
26. Ridley A, Cavanagh JB (1971) Lymphocytic infiltration in gliomas: evidence of possible host resistance. Brain 94: 117–124
27. 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
28. Rossi ML, Cruz-Sanchez FF, Hughes JT, Esiri MM, Coakham HB, Moss TH (1988) Mononuclear cell infiltrate and HLA-DR expression in low-grade astrocytomas: an immunohistological study of 23 cases. Acta Neuropathol 76:281–286
29. Rossi ML, Esiri MM, Cruz-Sanchez FF, Hughes JT, Coakham HB, Moss TH (1989) Characterization of the mononuclear cell infiltrate in 14 oligodendrogliomas: an immunohistological study. Br J Neurosurg (in press)
30. Schiffer D, Croveri G, Pautasso C (1974) Frequenza e significato degli infiltrati linfo-plasmacellulari nei gliomi umani. Tumori 60:177–184
31. 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
32. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA Jr, Shepard HM (1986) Recombinant human tumour necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230:943–945
33. Takeuchi J, Barnard RO (1976) Perivascular lymphocytic cuffing in astrocytomas. Acta Neuropathol (Berl) 35:265–271
34. Vilcek J, Palombella VJ, Henriksen-De Stefano D, Swenson C, Feinmman R, Hirai M, Tsujimoto M (1986) Fibroblast growth enhancing activity of TNF and its relationship to other polypeptide growth factors. J Exp Med 163:632–644
35. Von Hanwehr RI, Hofman FM, Taylor CR, Apuzzo MLJ (1984) Mononuclear lymphoid populations infiltrating the microenvironment of primary CNS tumours. Characterization of cell subsets with monoclonal antibodies. J Neurosurg 60:1138–1147
36. Vose BM, Moore M (1985) Human tumour-infiltrating lymphocytes: a marker of host response. Semin Hematol 22:27–40
37. Williamson BD, Carswell EA, Rubin BY (1983) Human TNF produced by human B cell lines: synergistic cytotoxic interaction with human IF. Proc Natl Acad Sci USA 80:5397–5401
38. Zucali JR, Elfenbein GJ, Barth KC, Dinarello CA (1987) Effects of human IL 1 and human TNF on human T lymphocyte colony formation. J Clin Invest 80:772–777

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