Mononuclear lymphoid populations infiltrating the microenvironment of primary CNS tumors

Characterization of cell subsets with monoclonal antibodies

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Mononuclear cell infiltrates are found to varying degrees in 30% to 60% of primary human central nervous system (CNS) gliomas. To explore the immunological importance of this, six operative glial tumors, eight non-glial tumors, and three normal brain specimens were studied. Utilizing an immunoperoxidase method, the authors examined frozen sections for lymphoid infiltrates expressing suppressor/cytotoxic and helper phenotypes, as identified with the Leu-1,2,3 monoclonal antibodies. Four of six gliomas demonstrated lymphoid infiltrates: three tumors exhibited a predominant suppressor/cytotoxic cell phenotype and the fourth showed mixed staining of suppressor/cytotoxic and helper cell phenotypes. Varying degrees of lymphoid infiltration characterized four out of eight non-glial primary CNS tumors. Two cases exhibited a prevalence of suppressor/cytotoxic phenotype cells, while two cases demonstrated a more heterogeneous pattern of phenotype expression. Normal brain sections revealed little or no evidence of mononuclear infiltrates. The immunobiological significance of these findings is discussed in the context of tumor-host interaction within the CNS.

KEY WORDS • monoclonal antibody • immunoperoxidase • brain tumor • T lymphocyte • T cell subset • tumor immunology

The host-mediated immune response to intrinsic central nervous system (CNS) tumors encompasses many interrelated humoral and cellular events representing varied modifications of normal immune function in the face of a neoplastic challenge. The presence of lymphoid cellular infiltrates associated with CNS gliomas was first appreciated by Bertrand and Mannen in 1960 and subsequently, in 1971, by Ridley and Cavanagh, who discussed the potential implications of lymphoid infiltrates as phenomena indicative of a host-mediated immune response to CNS neoplasia.

Viewed at one time from the perspective of absolute immunological privilege, the contemporary perception of the CNS as an immunological site with partial or selective privilege represents a transition generated in part by accumulating evidence that systemic immune effector cells enter the brain in substantial numbers under conditions that alter the integrity of the blood-brain barrier (BBB). These include structural neurovascular changes induced by trauma, neoplasia, and other processes such as experimental allergic (autoimmune) encephalitis.

Mononuclear infiltrates, comprising both perivascular and diffuse interstitial patterns of distribution, have been demonstrated within the parenchyma of human glial tumors in 30% to 60% of nearly 800 cases previously reviewed in the literature. The immunological significance of this phenomenon has been further elucidated by studies correlating the magnitude and intensity of the infiltrative lymphoid cell response with characteristics of tumor histology and survival in patients harboring CNS gliomas. In previous studies utilizing immunofluorescence techniques and rabbit antisera prepared against human T lymphocyte globulins appeared to represent a major subpopulation of mononuclear cell groups infiltrating gliomas.

The recent discovery that monoclonal antibodies recognize lymphocyte cell-surface antigens and the selective incorporation of these antibodies into refined immunohistochemical methods have permitted increasingly sensitive detection and precise definition of lymphocyte subsets within tissues, lending further clarification to their role in the cellular immune response.

As established in numerous studies, monoclonal anti-
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FIG. 1. Schematic representation correlating Leu-1,2,3 phenotype expression with patterns of lymphocyte differentiation and functional specialization. Symbols are as described in key.

bodies of the OKT and Leu designations permit the characterization of lymphocyte subsets through recognition of surface phenotype expression. The different phenotypes (antigenic surface membrane determinants) recognized by monoclonal antibodies may thus be viewed as reflecting stages of differentiation and functional immune specialization among differing lymphocyte subsets (Fig. 1).

Utilizing the Leu monoclonal antibodies and a modification of the avidin-biotin immunoperoxidase method, we examined the microenvironment of 14 human primary CNS neoplasms, including six gliomas and eight non-glial tumors, with respect to lymphoid cell infiltrates. Lymphocyte subsets, defined according to phenotype, were analyzed with reference to their projected functional role in the immunologically mediated host-tumor interaction and its modulation within the unique environment of the CNS. This paper presents our findings.

Materials and Methods

Materials

Tissue Samples. Fresh human glial and non-glial tumor specimens were obtained from 14 patients who had undergone operative biopsy. The samples included one astrocytoma, two malignant astrocytomas, three glioblastomas, five meningiomas, two medulloblastomas, and one malignant schwannoma. Control tissue specimens representative of normal brain were obtained from two fresh autopsy cases without underlying CNS disease, as well as from one case of closed head trauma. Cortical tissue in the latter instance originated from sites extrinsic to the underlying pathology.

Primary Monoclonal Antibodies. Lymphocyte subsets infiltrating tumor parenchyma were selectively identified with the antihuman leukocyte (Leu) monoclonal antibodies. The Leu-1 immunoglobulin (IgG2a), Leu-2a(IgG1), and Leu-3a(IgG1) monoclonal antibodies identify phenotypic markers for all mature lymphocytes (Pan-T), and for the suppressor/cytotoxic and the helper/inducer T lymphocyte subsets, respectively. The lyophilized antibodies were reconstituted to a final concentration of 10 µg/ml at a dilution factor of 1:20 with 1% sterile bovine serum albumin (BSA) in Tris buffer (pH 7.4).

Immunobiologically Active Reagents. Secondary biotinylated horse antibodies against mouse immunoglobulins were reconstructed from the lyophilisate and diluted with 1% sterile BSA in Tris buffer (pH 7.4) to a final concentration of 50 µg/ml. Avidin-biotin complexes conjugated to horseradish peroxidase were reconstituted and diluted in Tris buffer without BSA (pH 7.4).

Chromogenic Reagents. Chromogenic reagent, 3,3 diaminobenzidine (DAB), was prepared by suspending 6 mg of DAB in 9.9 cc of Tris buffer with subsequent

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* Monoclonal antibodies provided by Becton-Dickinson, Sunnyvale, California.
addition of 0.1 cc of \( \text{H}_2\text{O}_2 \) (3%) for immediate use. Cupric sulfate (\( \text{CuSO}_4 \)), 0.5% in 0.9% NaCl at 25°C, was then used for enhancement of staining intensity.

**Methodology**

**Preparation of Tissue Sections.** Fresh-tissue block specimens were processed within 6 hours of surgery: 7- to 10-mm tissue cubes were washed twice in RPMI (Roswell Park Memorial Institute) solution at 4°C, immersed in OCT freezing compound, and frozen in liquid nitrogen for storage at -70°C. Frozen specimen cubes were sectioned at 15-μ thickness, allowed to air-dry, and then immersed in fresh acetone (-20°C) for 20 to 30 seconds. Monoclonal antibody immunoperoxidase staining was performed within 1 week following preparation of frozen sections and acetone fixation.

**Avidin-Biotin Immunoperoxidase Staining Technique.** The avidin-biotin immunoperoxidase staining method is shown in Fig. 2. Frozen sections were fixed in fresh acetone (-20°C) for 10 minutes in order to facilitate preservation of tissue architecture. Glass wells were etched around the perimeter of individual tissue sections to permit efficient utilization of staining reagents and antibodies throughout the procedure. Slides were rehydrated and washed in phosphate-buffered saline (PBS, pH 7.4) for 8 minutes at 25°C. Following all PBS washes, slides were blotted dry around the etched wells to prevent dilution of antibodies and reagents. Normal horse serum at 1:20 dilution was then applied for 30 minutes to decrease nonspecific background binding of antibodies, followed by an 8-minute wash with PBS. Slide wells were then flooded with primary antibodies (10 μg/ml for 60 minutes) with secondary biotinylated horse antibodies against mouse immunoglobulins (50 μg/ml for 30 minutes), and finally with avidin-biotin complexes conjugated to horseradish peroxidase (for 60 minutes), with intervening 10-minute PBS washes terminating each of the above three steps in sequence. Slides were then treated in rapid succession with 5 minutes of DAB chromogenic reagent, a 10-minute tap-water rinse, 5 minutes of 0.5% \( \text{CuSO}_4 \) in 0.9% NaCl for color enhancement, and a final 8-minute wash in PBS. Nuclear counterstaining in hematoxylin and mounting in Aquamount concluded the procedure.

**Staining Controls.** As a control for nonspecific staining, primary antibody was omitted, with substitution by normal mouse ascites fluid or normal mouse serum. All other steps in the staining sequence were followed as described previously.

**Identification of Lymphocyte Subpopulations in Tissue Samples.** Sections from six primary glial tumors, eight non-glial CNS tumors, and three normal brain specimens were examined for the presence of mononuclear cell infiltrates using the above procedures. Representative tumor specimens are shown in Fig. 3. Immunoperoxidase-reactive lymphoid cell subpopulations within these heterogeneous mononuclear cell infiltrates were evaluated for the expression of phenotypic markers corresponding to the Pan T lymphocyte/mature thymocyte (E-rosette-positive cells), cytotoxic/suppressor, and helper/inducer subsets as detected and defined with the Leu-1,2,3 monoclonal antibodies, respectively. Immunoperoxidase staining responses were designated as specific if the reaction observed was intense, granular, and discretely focused within the membrane or cytoplasm of individual cells. The distribution of positively staining cells was described in terms of random, diffuse, or clustered patterns of orientation within the tissue architecture.

**Results**

A comparative profile of immunoperoxidase-reactive monoclonal cell subpopulations infiltrating the tumor microenvironment (characterized by lymphoid subset phenotype expression using the Leu monoclonal antibodies) is shown in Table 1. Immunoperoxidase staining of sections from normal brain specimens failed to detect mononuclear infiltrates in the perivascular spaces or in the cortical or subcortical parenchyma. There was little to no background staining and minimal cellular staining reaction was observed. In examining the 14 tumors comprising this series, semi-quantitative assessment of lymphocyte subsets identified by the various monoclonal antibodies was achieved by expressing their relative proportions as a percentage of the total heterogeneous population of nucleated cells present in a given microscopic field (that is, tumor cells, infiltrating mononuclear cells, or supportive cells). Infiltrating mononuclear cell populations representing 0% to 5%, 5% to 10%, and 10% to 25% of total cells in a given microscopic field were described in terms of slight,
FIG. 3. Photomicrographs of immunoperoxidase-stained tumor specimens demonstrating Leu antibody-positive lymphoid cell subpopulations infiltrating tumor parenchyma.  

Upper Left: Glioblastoma multiforme: Leu-2, × 312.  
Upper Right: Malignant meningioma: Leu-1, × 312.  
Lower Left: Malignant schwannoma: Leu-2, × 312.  
Lower Right: Fibroblastic meningioma: Leu-1, × 312.
TABLE 1
Monoclonal antibody-defined immunoperoxidase-positive lymphoid cell subsets in central nervous system tumors

<table>
<thead>
<tr>
<th>Tissue Sample No.</th>
<th>Diagnosis</th>
<th>Lymphoid Cell Staining*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Leu-1</td>
</tr>
<tr>
<td>glial tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>malignant astrocytoma</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>glioblastoma multiforme</td>
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<tr>
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</tr>
<tr>
<td>5</td>
<td>glioblastoma multiforme</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>astrocytoma</td>
<td>0</td>
</tr>
<tr>
<td>non-glial tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>fibroblastic meningioma</td>
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<td>meningothelial meningioma</td>
<td>R</td>
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<td>11</td>
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</tr>
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<td>12</td>
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<td>2</td>
</tr>
<tr>
<td>13</td>
<td>medulloblastoma</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>malignant schwannoma</td>
<td>1</td>
</tr>
<tr>
<td>control samples</td>
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<td></td>
</tr>
<tr>
<td>15†</td>
<td>closed head trauma</td>
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</tr>
<tr>
<td>16‡</td>
<td>grossly normal brain</td>
<td>0</td>
</tr>
<tr>
<td>17‡</td>
<td>grossly normal brain</td>
<td>0</td>
</tr>
</tbody>
</table>

* Semi-quantitative results were obtained with this method. The number of positive-staining cells is expressed as a percentage of the total cells present within a given microscopic field. 0 = no staining; R = rare positive-staining cells; 1 = slight staining (1% to 5%); 2 = moderate staining (5% to 10%); and 3 = marked intense staining (10% to 25%).
† Operative specimen from biopsy site representative of grossly normal brain extrinsic to region of trauma.
‡ Fresh autopsy specimens from normal brain without underlying pathology.

Moderate, and marked degrees of infiltration, respectively (Table 1).

Of the six glial tumors studied, two (Samples 1 and 6) showed essentially no evidence of mononuclear infiltrates or specific staining. Two additional tumor specimens (Samples 4 and 5) were positive for slight degrees of infiltration, with specific immunoperoxidase staining responses noted for the Leu-2 phenotype. One of these two specimens (Sample 4) also revealed an isolated and markedly positive reaction identifying a subset expressing the Leu-3 phenotype. The remaining two specimens (Samples 2 and 3) consistently exhibited a clear and, at times, intensely positive immunoperoxidase staining reaction along with the moderate to highest associated density of clustered and diffuse parenchymal mononuclear cell infiltrates predominantly composed of Leu-2-positive cells.

Eight non-glial primary CNS tumors were examined. Three were found to contain variable degrees of mononuclear infiltration with lymphoid cell subpopulations in two (Samples 7 and 14), characterized mainly by the Leu-1 and Leu-2 phenotypes. A prevalence of Leu-1 and Leu-3 phenotypes was noted in a third tumor (Sample 11). Moderately prominent infiltrates in a fourth tumor (Sample 12) also exhibited a predilection for expression of the latter phenotype pattern as detected by immunoperoxidase staining. The four remaining specimens either disclosed no evidence of mononuclear infiltration (Samples 8, 9, and 13) or involved the presence of rare perivascular lymphoid cells of the Leu-1 and Leu-2 phenotypes (Sample 10) with an absence of any apparent parenchymal infiltrates.

From an overall histological standpoint, the infiltrates observed consisted mainly of lymphoid-appearing cell types. Morphologically, the dominant cell resembled a small lymphocyte with a dense nucleus and scant cytoplasm. Frequent cells resembling microglial or macrophage populations were also encountered, although these did not appear to represent a majority of the cells present. In some cases, occasional clusters of transformed "blast-type" lymphoid cells reminiscent of activated lymphocytes were found.

Low levels of background and nonspecific cell staining were noted primarily with the Leu-3 monoclonal reagent; the Leu-1 and Leu-2 monoclonal reagents were consistently associated with minimal nonspecific cellular staining and little background. Slight background staining reaction was observed occasionally in control samples in which the primary specific antibodies were omitted, but this did not interfere with recognition of lymphocyte-related staining. Thus, in those instances where specific immunoperoxidase staining of a particular cell subpopulation was observed, the background and nonspecific cellular staining were slight and interpretation was straightforward.

In most cases, the mononuclear cell infiltrates were present diffusely throughout the tumor parenchyma, with occasional focal aggregates also noted. The different lymphocyte subsets, as revealed by staining with the three monoclonal antibody reagents, also showed a diffuse random distribution with the exception that Leu-2-positive cells were often concentrated in perivascular regions (as in Sample 5) or in intimate relation to small clusters of tumor cells. There was no morphological evidence of tumor cell destruction in these areas.

No characterization of the relationship, if any, between patterns of staining, histological observations, tissue distribution of mononuclear infiltrates, relative ratios of phenotype-defined cell subset densities, and the biological behavior or degree of malignant potential ascribed to each individual tumor was undertaken in this study.

Discussion

Mononuclear cell infiltrates constitute a phenomenon inherent to the microenvironment of primary human glial tumors, an observation previously reported in 30% to 60% of cases scrutinized by conventional histological techniques.4,10,29,31,42,44,46,49,54 Ample precedent exists from study of other disease entities to suggest an invasion of the CNS compartment by systemic immune effector cells.4,31,34,35,41,46,57,58 Mononuclear infiltrates may reflect an expression of host-tumor interaction, with inherent lymphocyte subpopulations po-
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tententially exerting functional impact in the mediation of immunosuppression within the CNS. Clearly, however, modification of any cellular host immune response to CNS neoplasms might occur as a consequence of unique limitations imposed by the CNS as a site of partial immunological privilege.3,30,41

This study represents an initial attempt, by means of monoclonal antibodies and an immunoperoxidase method, at in situ identification and characterization of lymphocytes within mononuclear cell populations infiltrating both human gliomas and non-glial primary CNS neoplasms. In the context of this series, our data confirm the generally accepted incidence of mononuclear infiltrates within gliomas, and tentatively reveal a predominance of suppressor/cytotoxic (Leu-2) lymphoid subpopulations invading the tumor parenchyma. It is assumed that patterns of phenotype expression observed within these heterogeneous mononuclear infiltrates may approximate the distribution of various functional lymphocyte subpopulations.

Highly sensitive monoclonal antibody/immunoperoxidase techniques may convey higher levels of resolution and specificity than previously attainable. Thus, these techniques can be a valuable adjunct in the detection of otherwise unapparent subtle parenchymal infiltrates. Prior studies conducted with more limited histological methods demonstrated mainly perivascular infiltrates, with mononuclear cells confined to regions within or adjacent to the Virchow-Robin spaces.10,33,54 Consequently, the issue of perivascular infiltrates, representative of either a passive or active process, was addressed as a finding compatible with some degree of immunological privilege attributable to the CNS.10,33 In contrast with these reports, our specimens contained predominantly parenchymal infiltrates, including lymphoid cell clusters in some instances surrounding nests of tumor cells. The presence of an infiltrative component with a histological distribution throughout the tumor parenchyma implies a more active migratory process, and is consistent with an evolving revision of prior concepts related to immunological barriers in the CNS.

The relative permeability of the neurovascular BBB resulting from alterations induced by neoplastic and non-neoplastic processes4,13,46,54 has been confirmed not only by the observation of mononuclear infiltrates in the case of both gliomas10,33,34,44,49,54 and experimental allergic encephalitis,34,35 but also by the discovery that glioma cells may enter the intravascular spaces.31 Furthermore, a recent study of mononuclear cell infiltration patterns within active lesions (plaques) from patients with chronic multiple sclerosis has disclosed a relationship between lesion progression in cases of multiple sclerosis and the presence of helper/inducer phenotype (T4+) T cells concentrated in large numbers throughout adjacent normal white matter.53 Suppressor/cytotoxic phenotype-positive cells (T8+) were restricted mainly to a perivascular distribution pattern within the margin of these lesions, while demyelination appeared to coincide with increased numbers of Ia+ cells (B lymphocytes and macrophages), especially at the center of most lesions. These results offer another example in support of a role for infiltrating systemic immune effector cells in the pathogenesis of both neoplastic and non-neoplastic CNS disease. In this regard, it is of interest that normal brain tissue appears free of detectable infiltrates, as determined in this and previous studies.63

The use of experimental animal models in an attempt to circumvent CNS barriers by performing subarachnoid lymphocyte infusions has failed to generate intraparenchymal or perivascular infiltration, in part due to failure to disrupt the neurovascular BBB.32 Thus, although some modification of the BBB may precede mononuclear cell migration into the CNS,4,13,31,42,46,60 the resultant immune effector response to primary CNS neoplasia may nevertheless have limited potential.1,3,30,32,33,41,46,54 This would appear to contrast with some non-neoplastic CNS disease entities, such as experimental allergic encephalitis or multiple sclerosis, that also involve an infiltrative response as well as heightened degrees of mononuclear cell infiltration observed within the microenvironment of some solid tumors outside the CNS.7,20,42,49 and their metastases to the CNS.52,33,44,64 In these instances, even greater disruption of the neurovascular BBB may be present than in the case of some primary CNS glial tumors. Furthermore, as a potential correlate to the suspected adverse immunobiological impact of BBB phenomena on primary glial tumors, it has been noted that, although lymphoid cells are frequently present within primary CNS tumor parenchyma, the direct inoculation of additional autologous leukocytes into CNS tumor beds may possibly enhance the survival of some glioma patients.51-53,67

In two series of glial and non-glial CNS tumors alike, the macrophage subpopulation (as judged primarily by morphological criteria) approached a fourfold prevalence over its lymphoid counterparts,54 and T cells constituted the majority of lymphocytes identified by immunofluorescence.49 Within sections from the gliomas in this study, however, the most prominent subpopulation of infiltrating mononuclear cells appeared lymphoid in nature, although frequent cells resembling macrophages, monocytes, and microglia were readily discernible.

Further observations arising from this study pertain to the occasional appearance of cell clusters reminiscent of transformed lymphoblasts or immunoblasts, especially in cases where they constituted part of an infiltrating cell population which preferentially expressed the Leu-2 phenotype. It is attempting to speculate that these may represent early matured T cells. Precedent for this observation exists in the demonstration that early T cells, at least as defined by high-affinity E-rosette positivity, can migrate into the CNS compartment during the active phase of experimental allergic encephalitis.58
A discrepancy in the semi-quantitative assessment of lymphocyte subpopulation ratios was observed in some cases; this is shown by the lower than expected number of cells positive for Leu-1 when contrasted with the sum of cell numbers staining for Leu-2 and Leu-3. Potential explanations that may be entertained include: variable expression of phenotypic determinants by lymphocyte subpopulations, expression of multiple determinants on less differentiated lymphocytes, early T cells, activated lymphoblasts, and possibly macrophages or monocytes displaying a Leu-2 and/or Leu-3 phenotype with little to no concurrent phenotype expression for Leu-1, and possible expression of cross-reacting antigens on neuronal, glial, or tumor cell elements.

Critical observations germane to the first two of these possibilities have been advanced in studies by Ledbetter et al., concerning the nature and distribution of phenotype-specific surface molecules on T lymphocytes as detected with the Leu antibodies. Discrete antigenic moieties have been identified for Leu-1, Leu-2, and Leu-3 (67,000, 32,000/43,000, and 55,000 relative molecular weights, respectively) with evidence of distinct patterns of expression on thymocytes and T lymphocytes. Specifically, expression of Leu-1 is a feature of the more mature thymocytes and peripheral T lymphocytes. Both Leu-2 and Leu-3 are variably but concurrently expressed on many thymocytes, with selective expression patterns becoming manifest with increasing cell differentiation.

Confirmation of attendant specificity with regard to the monoclonal antibody-directed immunoperoxidase staining was apparent in the paucity of background staining encountered with any of the monoclonal reagents in the samples of normal brain, and in the relatively low level of background staining present throughout the majority of tumor samples stained with the Leu-1 and Leu-2 reagents. The occasional presence of weak staining of tumor cells with the Leu-3 reagent posed no problem in distinguishing Leu-3-positive lymphocytes, which stained much more intensely.

Regarding the low-intensity staining reaction of tumor cells, it is conceivable that antigenic determinants on neural or glial CNS cell elements are altered or unmasked in some fashion as a result of neoplastic transformation, or that tumor cells themselves may express similar antigens, any of which might cross-react with monoclonal antibodies directed primarily at determinants on lymphoid cells. Clearly, it would be a mistake to assume that, simply because a particular monoclonal antibody demonstrates immunospecificity for a discrete antigenic determinant, it therefore must possess specificity for a particular cell type. Diverse cell types may display common determinants, and cross-reactivity of structurally unrelated determinants may occur. Corroborative evidence found in the literature supports the concept of shared or common antigenic determinants expressed concurrently on both CNS and lymphoid cells, among others. Cross-reactivity of the monoclonal antibody OKT-8 with oligodendrogial cells and of OKT-6 with Langerhans' cells in the skin, the detection of Thy-1 antigens on astrocytes, neuronal cells, and fibroblasts alike, and the verification in animal models of non-Thy-1 brain-associated T cell antigens with a possible regulatory role in T-helper cell/B cell interactions have all been reported. In addition, the expression of human leukocyte antigen (HLA)-DR determinants on long-term cultured glioma cell lines, and both HLA-DR and Thy-1 antigen expression within the microenvironment of human gliomas have been detected. The in vitro inhibitory influence of brain glycoprotein NSA$_3$ on lymphocyte proliferation and T-helper cell function (representative of a model of immunosuppression) provides further circumstantial data.

Correlation of cellular differentiation and relative functional immune specialization of lymphocyte subsets with patterns of phenotype expression as defined with monoclonal antibodies is well established. Nevertheless, in attempting to equate function with phenotype expression, it is clear that an individual phenotype may not always specifically subsume the same functional state — thus the qualified nature of any resulting conclusions deserves emphasis. A number of relevant examples are documented in the recent literature: normal lymphocytes and leukemic cells with an OKT-4 helper/inducer phenotype can participate in suppression of B cell differentiation under selected experimental conditions, and conversely, Leu-2 positive cells (also expressing a helper/inducer phenotype) may participate in helper cell responses in vitro, thus revealing some capacity for functional heterogeneity within presently defined lymphocyte subsets. Also, as has been previously discussed, non-lymphoid cells may express lymphoid phenotypes without functional import. Ultimately, the most evident limitation involves any prediction of possible functional impact exerted by Leu-2- or OKT-5/8-positive lymphocyte subpopulations, since these phenotypes entail the potential for both suppressor and/or cytotoxic activity.

A substantial body of literature presents conflicting insights on the relationship of mononuclear infiltrates to glial tumor evolution in terms of malignant behavior, tumor burden, histological cell types, and patient survival. Most studies have been unable to substantiate any link other than the presence and extent of lymphocyte invasion with enhanced survival, although in some series increased malignant potential appears paradoxically related to heightened levels of mononuclear infiltration despite the apparent associated evidence for prolonged survival. Some investigations of mononuclear infiltrates in systemic non-CNS neoplasia correlate infiltration with a lower incidence of metastasis, and possibly enhanced survival independent of other factors.

Previous attempts at classification of cell subsets within the mononuclear populations infiltrating gliomas have involved the distinction between lymphoid
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and non-lymphoid (monocyte/macrophage) components, as well as an assessment of Fc receptor expression.\textsuperscript{63,64} The presence of Fc receptor-positive lymphocytes, monocytes, and macrophages along with both tumor-associated and Fc receptor-bound immunoglobulins (IgG\textsubscript{1}, IgG\textsubscript{3}, IgA, and IgM), mostly in the form of antigen-antibody complexes, has been demonstrated in glial and non-glial primary CNS tumors.\textsuperscript{63,64} The significance of this observation remains to be clarified, but the presence of similar findings in non-CNS tumor models has been reported along with a tendency for these experimental systems to exhibit a relationship between the amount of tumor-associated immunoglobulin, the degree of host cell infiltration, and the expression of Fc receptor-bound immunoglobulin.\textsuperscript{23,30} In addition, attempts have been made to utilize expression of Fc receptors for IgG (so-called “T\textsubscript{g} cells”) as a marker for suppressor T cell subpopulations in human neoplasms.\textsuperscript{25,36} However, both lymphoid and non-lymphoid cells (including monocytes) contribute to this subpopulation, which may not always be equivalent to the suppressor/cytotoxic lymphocyte subsets defined by monoclonal antibodies.\textsuperscript{36,38} Nevertheless, T\textsubscript{g} subpopulation densities appear elevated both in the peripheral blood and in situ within a substantial series of non-CNS tumors.\textsuperscript{25} A suspected suppressor function associated with monocytosis in systemic neoplasms exhibiting depressed T cell activity \textit{in vitro} has also been described.\textsuperscript{66}

Although this present study represents one of the first monoclonal antibody-directed analyses of phenotypically defined lymphocyte populations infiltrating gliomas, mononuclear subpopulations in the peripheral blood of glioma patients have been subjected to extensive investigation.\textsuperscript{8,17,26} A marked decrease in functions related to cell-mediated immunity has been documented previously.\textsuperscript{5,11,65} Impaired immunocompetence, resulting from enhanced suppressive immunoregulatory cell function attributed to lymphocyte and monocyte subpopulations, is a well described phenomenon, as demonstrated in functional assays of peripheral blood leukocytes obtained from glioma patients.\textsuperscript{8,17} In some studies, impaired T cell function, as delineated with phytohemagglutinin (PHA) stimulation tests, appears associated with amplified levels of immunoregulatory activity exerted by suppressor cell subsets of probable monocytic and lymphoid origin, despite an apparent contribution toward deficiencies of generalized host immunocompetence attributable to T lymphocyte depletion in glioma patients.\textsuperscript{8} Alternatively, however, it has been proposed that CNS tumor-induced shifts in lymphocyte subpopulations, rather than potentially variable alterations in suppressor cell immunoregulatory function, may be largely responsible for the impaired immunocompetence observed in many glioma patients.\textsuperscript{45} No correlation of decreased T cell function with the detection of soluble immune complexes in patients' sera could be verified,\textsuperscript{45} in contrast to other studies demonstrating the presence of circulating humoral suppressor factors or antigen-antibody complexes.\textsuperscript{5,9,11,28,65}

Previous investigators have suggested the presence of “glioma-associated antigen” overload arising as a result of rapid tumor growth and overwhelming tumor burden, leading to immune complex formation and the activation of mechanisms producing immunosuppression with resultant immunological paralysis.\textsuperscript{34} The induction of suppressor cell populations could potentially reflect the presence of immune complexes associated with Fc receptor-bearing lymphocytes (including T\textsubscript{g} cells) infiltrating tumor,\textsuperscript{33,25,63,64} as well as potential interaction between tumor cell-antibody complexes and respective Fc receptor-bearing subpopulations, including macrophages or monocytes.\textsuperscript{18,66} Cells expressing Fc receptors appear necessary in some models for induction of immunosuppression leading to tumor enhancement.\textsuperscript{48} Circulating tumor cells may also be implicated in triggering immunosuppression,\textsuperscript{9} and infiltrating effector cells bearing Fc receptors that have attached antitumor immunoglobulins may form complexes with tumor antigens, followed by shedding which may in turn lead to further formation of soluble immune complexes and increased immunosuppression.\textsuperscript{33,25,63,64}

Indeed, it has been postulated that one role of T\textsubscript{g} cells may be in the delivery of a suppressor signal\textsuperscript{59} in response to any of the above-mentioned conditions favoring an increase in immunoregulatory suppressor cell functional subsets, ultimately resulting in decreased immunocompetence, the induction of tolerance, and the undesired effect of an active enhancement of neoplastic progression.\textsuperscript{5,9,18} In a recent study assessing Fc receptor-positive mononuclear cell subset patterns, including their presence and potential functional activity, T\textsubscript{g} subpopulations appeared to be increased in the peripheral blood of glioma patients, perhaps implying enhanced suppressor subset activity.\textsuperscript{17} Concurrent studies completed in our laboratory have characterized suppressor/cytotoxic (OKT-5/8) and helper/inducer (OKT-4) phenotype-defined mononuclear cell subsets in the peripheral blood utilizing monoclonal antibodies with flow cytometric-immunofluorescence analysis techniques.\textsuperscript{26} These techniques should be applied prospectively to future patients with gliomas.

Our studies have yielded some results that do not appear compatible with the above findings;\textsuperscript{17} a partial explanation may reside in previous observations that subpopulations defined by Fc\textsubscript{g} and Fc\textsubscript{m} receptors are not necessarily equivalent to monoclonal antibody phenotype-defined subsets.\textsuperscript{38} Thus, increased T\textsubscript{g} subsets detected by analyzing the expression of Fc receptors for IgG (presumptive suppressor cells)\textsuperscript{17} may fail to reflect a shift in the dynamics of OKT-5/8- or Leu-2-defined suppressor/cytotoxic subsets as previously detected in this laboratory; namely, a decrease of this subpopulation in the peripheral blood of patients with malignant gliomas.

In attempting to arrive at a plausible correlation of the decrease in peripheral blood suppressor/cytotoxic
cells (Leu-2-positive) with the presence of increased suppressor/cytotoxic cells (Leu-2-positive) infiltrating glial tumor parenchyma, one likely explanation emerges. The active migration of systemic effector cells into a target organ, such as the CNS, as a neoplastic site might lead to selective depletion of active functional subsets from the peripheral pool; the potential for an ensuing concentration effect at the tumor site could subsequently result in a predominant tendency for the expression of a distinct and specialized immunological function,23 in this case resulting in the creation of a favorable niveau for tumor enhancement. In view of our demonstration confirming the presence of predominantly suppressor/cytotoxic phenotype expression among cellular subsets infiltrating the microenvironment of glial neoplasms, this study provides evidence in support of a potential functional role for cellular effector mechanisms active in the mediation of immunosuppression, with resultant modulation of the host response to a neoplastic challenge originating within the CNS.

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