Immunoglobulin gene rearrangement and antigenic profile confirm B cell origin of primary cerebral lymphoma and indicate a mature phenotype

W J SMITH,* J A GARSON,† S P BOURNE,‡ J T KEMSHEAD,† H B COAKHAM‡

*From the *Department of Histopathology, University College Medical School, †ICRF Oncology Laboratory, Institute of Child Health, London, and the ‡Department of Neurosurgery, Frenchay Hospital, Bristol*

SUMMARY Six cases of primary cerebral lymphoma were immunophenotyped and analysed by Southern blotting to determine the clonality and lineage of these neoplasms. Molecular analysis showed that they were of B cell origin, and the rearrangement of both heavy and light chain immunoglobulins in malignant cells showed that they were monoclonal populations of mature B cells.

The characterisation of the genetic configuration of the immunoglobulin genes in these lymphomas is important because the ability to distinguish between primary lymphoma of the central nervous system and other malignant cerebral tumours has important implications for treatment and survival.

Primary cerebral lymphoma is relatively rare, comprising between 0.3 and 1.5% of all intracranial tumours.¹ Until recently, several diagnostic labels were applied to this tumour, including reticulum cell sarcoma and microglioma. The precise cell of origin of primary cerebral lymphoma is a subject of controversy, although an association between these neoplasms and non-Hodgkin's lymphoma has been acknowledged.² The ability to distinguish between primary lymphoma of the central nervous system (CNS) and other malignant cerebral tumours has clinical consequences, as many cerebral lymphomas are radiosensitive.³ The use of monoclonal antibodies has facilitated such differential diagnosis in certain cases.⁴ An immunohistochemical study of 20 cerebral lymphomas showed binding by the monoclonal antibody B1, which recognises the antigen CD20, indicating that they were B cell malignancies.⁴ The presence of reactive T cells in cerebral lymphomas, however, may lead to difficulty in diagnosis if the immunohistochemical data are not clear.

The use of molecular techniques to determine the lineage and clonality of lymphoproliferative conditions has become more widespread in the past few years due to the availability of recombinant DNA probes encoding the immunoglobulin and T cell receptor genes.⁵ These genes are organised in the genome in a segmental fashion and undergo somatic rearrangement during commitment to the B or T cell

lineage.⁶ Each lymphocyte undergoes a specific rearrangement of its immunoglobulin or T cell receptor genes, which is unique to the cell. Consequently, if a particular cell undergoes a clonal expansion, the whole clone can be identified by its genetic configuration using the Southern blot technique. The ability to distinguish between monoclonal and reactive proliferations of lymphocytes is of considerable benefit in the diagnosis of lymphoma.

We analysed six cases of primary cerebral lymphomas by immunophenotyping and by Southern blot analysis to determine the clonality and lineage of these neoplasms.

Material and methods

Cases were diagnosed as primary cerebral lymphoma on the basis of histological and immunohistochemical studies. DNA was extracted from snap frozen biopsy specimens, in which the presence of lymphoma had been confirmed by standard methods.⁷ After incubation with a restriction enzyme (EcoRI, HindIII, or BglII), the restriction fragments were separated according to size by gel electrophoresis and transferred to a nylon membrane (Gene-Screen Plus, DuPont) by Southern blotting.⁸ Recombinant DNA probes (table 1)⁹⁻¹¹ were radiolabelled with ³²P-dCTP by oligo labelling¹² and hybridised to the filter. After stringent washing the filter was exposed to pre-fogged x-ray film at -70°C for between two and four days.

The immunohistochemical studies were carried out

Table 1 Recombinant DNA probes

Probe	Code name	Reference
Heavy chain, joining region (Jh)	C76R51A	7
κ light chain, constant region (Ck)	pUCR17	9
λ light chain, constant region (C λ)	pUC λ 5	10
T cell receptor β chain (C β)	C β 2	11

Table 2 Monoclonal antibodies

Monoclonal antibody	Tissue specificity	Source/Reference
2D1	Pan-leukocyte and lymphoma	Dr P Beverley ¹³
LC (PD7/26 & 2B11)	Pan-leukocyte and lymphoma	Dakopatts ¹⁴
B1	Pan-B cell (CD20)	Coulter Clone ¹⁵
FMC7	Mature B cell	Sera Lab ¹⁶
UCHT1	Pan-T cell (CD3)	Dr P Beverley ¹⁷
Leu 2a	Suppressor/cytotoxic T cells (CD8)	Becton Dickinson ¹⁸
Leu 3a	Helper/inducer T cells (CD4)	Becton Dickinson ¹⁸
Leu 7	Natural killer cells and large granular lymphocytes	Becton Dickinson ¹⁹
UJ13A	Pan-neuroectodermal	Dr JT Kemshead ²⁰

on frozen and fixed tissues using the antibodies detailed in table 2.¹³⁻²⁰ Frozen sections (6 μ m) were hydrated for five minutes in phosphate buffered saline (PBS) on gelatine coated slides. After removing excess PBS 100 μ l of diluted monoclonal antibody was applied and incubated for 30 minutes at room temperature. Non-immune ascitic fluid was used as a negative control. Two washes in PBS were followed by a 30 minute incubation with 100 μ l of fluorescein conjugated, affinity purified, goat anti-mouse immunoglobulin. Unbound conjugate was removed by two washes in PBS and the sections were mounted in 90% glycerol/10% PBS for examination by epifluorescence microscopy. Haematoxylin and eosin staining was performed on subsequent sections to facilitate the orientation and interpretation of the fluorescence results.

Formalin fixed paraffin embedded tissue sections were dewaxed and endogenous peroxidase activity

inhibited by immersion in 0.5% hydrogen peroxidase in methanol for 30 minutes. The protocol outlined for frozen sections was followed, except that the fluorescein conjugate was replaced by peroxidase conjugated goat antimouse immunoglobulin. The bound antibody was visualised by incubation with diaminobenzidine. Sections were counterstained with Harris's haematoxylin, dehydrated through an alcohol series, and mounted.

Results

Immunostaining of all the biopsy sections from this series of cerebral malignancies with the pan-leucocyte antibody 2D1 and the pan-B cell reagent B1 confirmed that they were B cell populations (table 3). Membrane rather than cytoplasmic binding was observed. In all the samples tested with FMC7 20-70% of the cells expressed the antigen recognised by this antibody. A sparse population of cells (5%) bound the anti-T cell antibodies. None of the cells was immunoreactive with the monoclonal antibodies that recognise antigens expressed by natural killer cells (Leu7) or neuroectodermal tissue (UJ13A).

The molecular analysis of the six primary cerebral lymphomas (table 4) confirmed the immunohistochemical data which showed that they were B cell populations (table 4). The demonstration of a population of cells all possessing the same rearrangement of the Jh locus indicates the presence of a monoclonal B cell population. The novel restriction fragments were not generated by DNA polymorphisms as they were detected in all three restriction digests analysed for each case. The germline fragments which hybridised to the Jh probe originated from the normal cells in the biopsy specimen or, in case B7, the non-rearranged Jh allele in the neoplastic clone. In all cases, with the exception of B7, both alleles of the immunoglobulin heavy chain gene had undergone somatic recombination. The intensities of the non-germline bands on the autoradiographs indicated that the neoplastic clones comprised a high proportion of the cell populations examined.

Immunohistochemical study of these cases did not include an analysis of the immunoglobulin light chains

Table 3 Immunophenotype of cases

Case	2D1	LC	B1	FMC7	UCHT1	Leu2A	Leu3a	Leu7	UJ13A
B5	+	+	+	NT	-*	-	-	-	-
B6	+	+	+	+	-*	-	-	-	-
B7	+	+	+	+	-*	-	-	-	-
B8	+	+	+	+	-*	-	-	-	-
B9	+	+	+	+	-*	-	-	-	-
C1	+	+	+	NT	-*	-	-	-	-

+ = positive; - = negative; NT = not tested; -* = scattered infiltrate of UCHT1 positive cells (<5%) present.

Figure Illustration of immunoglobulin heavy chain joining region (*Jh*) gene rearrangement and germline configuration of genes encoding T cell receptor β chain constant region (*C β*) in cases B6, B8, B9 and C1 of primary cerebral lymphoma. G = germline; R = rearranged; Kb = sizes of molecular weight markers in kilobases.

present on the cell surface. Rearrangement of the Ck genes was detected in four of the five cases in which it was possible to examine the configuration of the light chain genes (table 4). Clonal deletion of the Ck genes had probably occurred in case B8. The patterns of hybridisation to the *Jh* probe in this case indicated that about 80% of the cell population was monoclonal while the remaining 20% possessed germline immunoglobulin heavy chain genes. On probing with the Ck probe, the band representing the germline fragment was about 20% of the intensity which would have been observed if all the cells had possessed non-rearranged Ck genes. No other restriction fragments complemen-

tary to the Ck probe were observed. These data suggest that the malignant cells (80%) had undergone a clonal deletion of their Ck genes.

The *C λ* locus is highly polymorphic, and comparing its configuration in normal and putative lymphoma samples from the same patient facilitates the identification of a rearrangement at this locus.¹⁰ Configurations of the *C λ* loci similar to those of published germline patterns¹⁰ were detected in four of five samples (table 4). In case B8 a restriction fragment which hybridised to the *C λ* probe was observed in addition to the germline fragments. Although no control tissue from this patient was available, the

Table 4 Genotypic results

Case No	<i>Jh</i>			<i>Ck</i> <i>BgIII</i>	<i>Cλ</i> <i>HindIII</i>	<i>Cβ</i>	
	<i>EcoRI</i>	<i>HindIII</i>	<i>BgIII</i>			<i>HindIII</i>	<i>EcoRI</i>
B5	G + R/R	G + R/R	ND	Insufficient DNA			
B6	G + R/R	G + R/R	G + R/R	G + R/R	G	G	G
B7	ND	G + R	G + R	G + R/R	G	G	G
B8	G + R/R	G + R/R	G + R/R	G + D	G + R	G	G
B9	G + R/R	G + R/R	G + R/R	G + R	G	G	G
C1	G + R/R	G + R/R	G + R/R	R/R	G	G	G

G = germline genetic configuration; R = rearranged genetic configuration; D = deleted; ND = not done.

Table 5 Clinical details

Case	Age/sex	Site	Original histological diagnosis	Immunological diagnosis	Genetic diagnosis	Radiation	Survival
B5	77/F	Corpus callosum	Lymphoma	B cell lymphoma	B cell lymphoma	No	1 month
B6	69/M	Temporal	?Lymphoma ?secondary carcinoma	B cell lymphoma	B cell lymphoma	Yes	2 years
B7	58/F	Temporal	Undifferentiated metastasis	B cell lymphoma	B cell lymphoma	No	2 months
B8	65/F	Fronto-temporal	Lymphoma	B cell lymphoma	B cell lymphoma	No	3 weeks
B9	68/F	Frontal	?Lymphoma secondary carcinoma	B cell lymphoma	B cell lymphoma	Yes	3 weeks 4 years
C1	46/F	Occipito-parietal	Lymphoma	B cell lymphoma	B cell lymphoma	Yes	2 years, alive

presence of this fragment in combination with the apparent clonal deletion of the Ck locus indicates a rearrangement at the C λ locus.

The state of the genes encoding the constant region of the β chain of the T cell receptor was examined in each case with two restriction enzymes EcoRI and HindIII. There was no evidence of genetic rearrangement at this locus in any sample (table 4).

Discussion

The combination of immunohistochemical and genetic studies on this series of primary cerebral lymphomas confirmed that they were B cell malignancies. The rearrangement of both the heavy and light chain immunoglobulin genes in these cells showed that they were monoclonal populations of mature B cells. Despite the presence of reactive T cells, recognised by anti-T cell antibodies, no evidence of a monoclonal T cell population was identified by molecular techniques. Although the Southern blotting technique is relatively time consuming, it is useful in cases in which the clonality or lineage of a lympho-proliferative population is not clarified by immunohistochemical techniques.

In our experience the immunohistochemical demonstration of surface immunoglobulin light chains in cerebral tissue is unsatisfactory. The presence of immunoglobulin released by necrotic tissue leads to non-specific reactivity of the anti-light chain antibodies. "Freeze and thaw" and "crush" artefacts adversely affect the interpretation of these immunohistochemical data. Immunoglobulin gene rearrangement occurs during development in a hierarchical manner: immunoglobulin heavy chain genes are rearranged before light chain genes.²¹ The hierarchy extends to light chain genes: κ chain gene rearrangement precedes that of λ . The demonstration of light chain gene rearrangement in the primary cerebral lymphomas indicates that they possess a relatively

mature genotype. This agrees with the immunophenotypic data as the monoclonal antibody FMC7 recognises an antigen expressed by mature B cells.¹⁶

Studies on non-Hodgkin's lymphoma biopsy specimens from various sites have shown that most lymphomas that were immunoreactive with the monoclonal antibody B1 possessed rearranged immunoglobulin genes.^{22,23} This was true even if the percentage of cells expressing the antigen was low. Molecular analysis has proved to be of particular value in the characterisation of "null" non-Hodgkins lymphomas, most of which possess rearranged immunoglobulin genes.^{24,25} The data presented in this paper on primary cerebral lymphomas correlate with those already published on non-Hodgkins lymphomas.

The clinical consequences of identifying cerebral lymphoma are illustrated by cases B6 and B9 (table 5). The original histological diagnoses could not differentiate between lymphoma and secondary carcinoma. Immunophenotyping and genetic analysis confirmed that lymphoma was present which proved to be radiosensitive in these cases. Similarly, genetic and immunological analysis of case B7 showed the presence of B cell lymphoma rather than an undifferentiated metastasis. The correct diagnosis of this case was made retrospectively (on frozen tissue) as part of an immunohistological review of undifferentiated tumours. The patient had not received radiotherapy and died two months after biopsy. This contrasts with the relatively longer survivals of those patients who had received radiotherapy (table 5).

The characterisation of the genetic configuration of the immunoglobulin genes in these lymphomas would be of benefit in the event of a patient developing a further tumour. If the new neoplasm possessed a genetic configuration identical with that of the original lymphoma, it would indicate the re-emergence of the original B cell clone.

In conclusion, we have shown that the primary cerebral tumours studied in this series by immun-

ological and genetic analysis were B cell malignancies, arrested at the mature end of the B cell differentiation pathway. These methods provide the neuropathologist with information which may have important clinical implications.

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Requests for reprints to: Dr W J Smith, Department of Histopathology, School of Medicine, University College London, University Street, London WC1E 6JJ, England