Identification of three antigens in human brain associated with similar antigens on human leukaemic cells

Elizabeth J. QUACKENBUSH, Tony F. CRUZ, Mario A. MOSCARELLO and
Michelle LETARTE*

*Divisions of Immunology and Biochemistry, Research Institute, The Hospital for Sick Children, Toronto,
Ontario, Canada M5G 1X8*

(Received 18 May 1984/Accepted 19 September 1984)

Monoclonal antibodies prepared against a non-T and non-B acute-lymphocytic-leukaemia cell line were tested for reactivity against human brain tissue. Several of the monoclonal antibodies were found to react specifically with brain fractions. Three antigens, 44H4, 44D7 and 44D10, were identified in white matter. Although 44D10 was absent from grey matter, the levels of 44H4 and 44D7 antigens present in grey matter were 2- and 4-fold higher respectively than in white matter. Fractionation of white matter indicated that all three antigens were absent from the multilamellar compact myelin, but associated with a membrane fraction of higher density. All three antigens, which required detergent for solubilization from the membranes, were purified by affinity to monoclonal antibodies and/or were analysed by immunoblotting. The 44H4 and 44D10 antigens were single polypeptide chains with M_r 94000 and 80000 respectively when resolved by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Monoclonal antibody 44D7 reacted with a complex of a M_r > 120000 under non-reducing conditions in the presence of sodium dodecyl sulphate. This complex dissociated on reduction into four bands with M_r values of 80000, 57000, 47000 and 41000. The brain antigens are present on proteins similar to, or identical with, those isolated from acute-lymphocytic-leukaemia cells.

Several cell-surface proteins of lymphocytes have been shown to share antigenic determinants with membrane proteins of brain. Thy-1 glycoprotein of M_r 25000 is found in large amounts in brain and thymus of rat and mouse (Reif & Allen, 1964; Letarte-Muirhead *et al.*, 1975; Barclay *et al.*, 1975). Although expressed on a small subpopulation of human lymphocytes, Thy-1 is present on human brain (Dalchau & Fabre, 1979; McKenzie & Fabre, 1981). Another antigen, W3/13, a glycoprotein of M_r 95000, is also

Abbreviations used: MAG, myelin-associated glycoprotein; ALL, acute lymphocytic leukaemia; MAb, monoclonal antibody; c.r.i.a., cellular radioimmunoassay; IgG and IgM, immunoglobulins G and M; RAM-Fc, F(ab')₂ pepsin fragment of rabbit IgG produced against the purified papain Fc fragment of mouse IgG and purified by affinity to mouse IgG; RAM-IgG, rabbit IgG anti-mouse IgG; SDS, sodium dodecyl sulphate; phosphate-buffered saline, 0.01 M-phosphate buffer, pH 7.4, containing 0.15 M-NaCl, 1 mM-CaCl₂, 0.5 mM-MgCl₂ and 1 mM-KCl.

* To whom correspondence and reprints should be addressed.

expressed on both thymus and brain of rat (Williams *et al.*, 1977; Standring *et al.*, 1978). An antigen of similar M_r , shared between human T-lymphocytes, granulocytes and brain, and defined with monoclonal antibody F10-44-2, was originally thought to be homologous to the W3/13 antigen (Dalchau *et al.*, 1980). However, more recent data have indicated that F10-44-2 antigen is biochemically distinct from W3/13 (McKenzie *et al.*, 1982). HNK-1 (Leu-7), a monoclonal antibody reactive with a subpopulation of human peripheral blood lymphocytes with natural killer-cell activity, has been reported to react with the white matter of the central and peripheral nervous systems (Schuller-Petrovic *et al.*, 1983; McGarry *et al.*, 1983). It has been shown to react with a protein identical, or highly cross-reactive, with myelin-associated glycoprotein (MAG; M_r 108000) (McGarry *et al.*, 1983).

With the exception of Thy-1 (Campbell *et al.*, 1981; Cotmore *et al.*, 1981), very little is known about the chemical nature of the other antigenic components shared between brain and lympho-

cytes. Monoclonal antibodies reactive with brain antigens constitute excellent tools for the purification and biochemical characterization of these molecules and as immunocytochemical probes for their localization within central and peripheral nervous systems.

In the present investigation we studied the reactivity of several monoclonal antibodies prepared against a non-T, non-B ALL-cell line with human brain tissue. The reactivity of these antibodies with normal and malignant haematopoietic cells has been demonstrated (Quackenbush & Letarte, 1985). Our results indicate that the three antigens characterized biochemically in brain white matter are found on proteins similar to those present on ALL cells.

Materials and methods

Fractionation of human white matter

Fractionation of white matter represents an extension of a previously described procedure (Lowden *et al.*, 1966). Briefly, white matter (30g) dissected from human brain was homogenized in cold 0.25M-sucrose with a motor-driven glass/Teflon homogenizer, diluted to 250ml and centrifuged as shown in Scheme 1. Fraction P₂ was resuspended in 120ml of 0.88M-sucrose and divided into 10ml portions, which were layered with

Scheme 1. Fractionation of human brain white matter

3ml of 0.25M-sucrose and centrifuged at 100000g for 60 min. After centrifugation, the 0.25/0.88M-sucrose interface was removed for the preparation of the myelin fraction as described by Lowden *et al.* (1966). The pellet, P₃, was resuspended in 0.25M-sucrose and layered on a discontinuous gradient, formed by layering 16ml each of 1.2M-, 1.0M- and 0.8M-sucrose. After centrifugation at 96000g for 90 min, the material present at the interfaces was collected, diluted with water, and centrifuged at 100000g for 30 min. The samples were resuspended in 0.25M-sucrose and the protein content determined (Peterson, 1979).

Monoclonal antibodies: production and purification

Monoclonal antibodies (MAb) (Table 1) 44D7, 44D10, 44H4, 44E3, 44H7 and 44H9 were obtained by immunization of Balb/c mice with cells from the non-T, non-B ALL-cell line (HOON cells, Okamura *et al.*, 1984) and by fusion with SP2/0-Ag14 non-secretory myeloma cells (Quackenbush & Letarte, 1985). MAb 50B4 and 50C7 were obtained similarly, but after immunization of Balb/c mice with glycoproteins prepared from chronic-lymphocytic-leukaemia cells (Letarte *et al.*, 1984). All hybridoma lines have been cloned and re-cloned, and have maintained a stable production of IgG.

For the preparation of MAb affinity columns, the IgG fractions were obtained by (NH₄)₂SO₄ (45% satd.) precipitation of the hybridoma culture supernatants, followed by DEAE-Sephadex A-50 chromatography for the γ_1 antibodies (44D7, 44H7, 44E3) or by affinity to Protein A-Sepharose (Pharmacia) for the antibody 50B4 of γ_2a subclass. The purified IgG fractions were coupled to CNBr-activated Sepharose 4B at a ratio of 2mg of protein/ml of beads according to the manufac-

Table 1. Monoclonal antibodies to ALL cells tested for reactivity with human brain

M_r values were estimated by SDS/polyacrylamide-gel electrophoresis under reducing conditions [10 or 12.5% (w/v) acrylamide]. Data for ALL cells, except those for 44H9, are taken from Quackenbush & Letarte (1984).

MAb	M_r of antigen	
	From ALL cells	In brain white matter
44H4, 44E3	94000	94000 (major)
		90000 (minor)
44D7, 44H7	90000	80000
	57000	57000
	47000	47000
44D10, 50B4, 50C7	41000	41000
	85000	80000
	51000	Undetectable
44H9		

turer's (Pharmacia) instructions. The human IgM antibody to myelin-associated glycoprotein was generously provided by Dr. P. Braun, Department of Biochemistry, McGill University, Montreal, Canada. The F10-44-2 IgG was generously given by Dr. J. Fabre, Blond McIndoe Centre for Medical Research, East Grinstead, Sussex, U.K.

Cellular radioimmunoassay

The presence of antigens reactive with different MAbs in brain homogenates, subcellular fractions, soluble extracts and purified fractions was assayed by inhibition of the appropriate c.r.i.a. (Letarte *et al.*, 1980). In most experiments, brain fractions were incubated for 1 h in 1% Triton X-100 before assay in order to reveal possibly hidden antigenic sites of membranes. Briefly, serial dilutions in phosphate-buffered saline containing 0.5% bovine serum albumin of brain fractions to be tested (100 μ l) were incubated for 16 h at 4°C with an appropriate dilution of each MAb (100 μ l). After 4 min centrifugation at 10000g, 25 μ l aliquots, in triplicate, were transferred to microtitre plates, and incubated for 2 h at 4°C with 5×10^5 glutaraldehyde-fixed HOON cells (25 μ l). Residual MAb bound to the target cells was detected by incubation for 2 h at 4°C with 125 I-RAM-Fc (25 ng/well; 100000 c.p.m.) (Letarte *et al.*, 1980). One unit of antigenic activity is defined as the dilution giving 50% inhibition of binding of the unabsorbed antibody to the target cells. Total units of activity per fraction were calculated for each MAb assay. Specific activity is expressed in units/mg of protein and yield is estimated by comparison of units of activity present in a given fraction with that of the original brain homogenate.

Immunochemical identification of the antigens

Samples containing equal amounts of protein (80 μ g) from white matter or from the ALL-cell line, HOON, were solubilized with 1% SDS containing 5% β -mercaptoethanol and separated on 10% (w/v) polyacrylamide gels (Maizel, 1971). The proteins were transferred to cellulose nitrate sheets and the antigens identified by reaction with the appropriate monoclonal antibody essentially as described by Towbin *et al.* (1979). The blots were allowed to react for 16 h at 4°C with a 100-fold dilution of the hybridoma culture supernatants containing MAb. The blots were washed and then allowed to react with 5 μ g (2×10^7 c.p.m.) of either 125 I-RAM-Fc or 125 I-goat anti-human IgM (Tago, Burlingame, CA, U.S.A.).

Purification of antigens by antibody affinity chromatography

The antigens 44D7, 44D10 and 44H4 were

purified by MAb affinity chromatography. Briefly, fraction P₃ (Scheme 1) was solubilized in a final concentration of 1.5% Triton X-100 in 0.05 M-Tris (pH 8.0)/0.04% NaN₃ supplemented with 0.001 M-phenylmethanesulphonyl fluoride and 0.01 M-iodoacetamide (in order to alkylate any free thiol groups) for 2 h at 4°C. A soluble fraction was recovered by centrifugation at 100000g for 60 min. The soluble extract (65 ml) was fractionated sequentially through columns of non-immune IgG-Sepharose, 44H7-IgG (or 44D7-IgG)-Sepharose, 50B4-IgG-Sepharose and 44E3-IgG-Sepharose. Columns were washed with 0.05 M-Tris buffer, pH 8.0, containing 0.15 M-NaCl, 0.04% NaN₃ and 0.1% Triton X-100.

Antigens bound to the columns were eluted with 0.05 M-diethylamine, pH 11.2, containing 0.1% Triton X-100 and neutralized with 1 M-glycine/HCl and concentrated 5–6-fold. All fractions were assayed for antigenic activity by c.r.i.a. and for protein content (Peterson, 1979). Portions (25 μ g) of affinity-purified antigens were labelled with 125 I by using 15 μ g of Iodogen (Pierce) (Markwell & Fox, 1978); free radioactivity was removed by Sephadex G-50 chromatography.

Radiolabelled antigens were immuno-precipitated as follows. Portions were incubated with RAM-IgG-Sepharose (1 h; 4°C) before incubation with saturating levels of MAb culture supernatants for 16 h at 4°C and RAM-IgG-Sepharose (1 h; 4°C). Beads were washed three times and eluted with 2% SDS, for 5 min at 100°C. Eluates were counted for radioactivity and subjected to SDS/polyacrylamide-gel electrophoresis (Maizel, 1971) in the presence or absence of dithiothreitol. All samples were alkylated with iodoacetamide before electrophoresis. Autoradiography was performed with XR-1 film and Du Pont Cronex Hi-plus intensifying screens (Swanstrom & Shank, 1978).

Electron microscopy

Sucrose gradient fractions were centrifuged for 30 min at 15000g and the pellets were fixed with 2% glutaraldehyde in 0.1 M-phosphate buffer, pH 7.2, for 16 h at 4°C. The samples were stained with uranyl acetate and lead citrate and the sections were viewed with a Philips 4000 electron microscope.

Results

Reactivity of monoclonal antibodies to ALL cells with human grey and white matter

Several monoclonal antibodies prepared against HOON, a non-T, non-B ALL-cell line (Okamura *et al.*, 1984) were compared for their reactivity towards grey and white matter of human brain.

Table 2. *Comparison of antigenic activity in normal human brain grey and white matter*

One unit of activity is defined as the dilution of homogenate or fraction required to inhibit 50% of the binding of the MAb to HOON target cells in the appropriate c.r.i.a. Abbreviations used: und, undetectable levels of antigenic activity, PBS, phosphate-buffered saline.

MAb	Specific antigenic activity (units/mg of protein)			
	Grey matter		White matter	
	In PBS	In Triton X-100	In PBS	In Triton X-100
44H4	2.3	2.9	1.1	1.6
44D7	1.1	2.6	0.3	0.6
44D10	und	und	1.1	1.4
44H9	und	und	und	und

Table 3. *Distribution of the three antigens 44H4, 44D7 and 44D10 during differential centrifugation of brain-white-matter homogenate*

Antigen ...	Total antigenic content per fraction					
	44H4		44D7		44D10	
	Units	Yield (%)	Units	Yield (%)	Units	Yield (%)
Fraction						
Homogenate	6500	100	2000	10	3650	100
P ₁	1500	23	300	7	372	10
S ₁	246	4	656	33	1886	52
P ₃	4800	74	1320	66	1980	54
Myelin	348	5	120	6	36	1

The antibodies are listed in Table 1. Previous studies have indicated that MAb 44E3 and 44H4 recognize the same antigen on ALL cells, a polypeptide of M_r 94000. MAb 44H7 and 44D7 both reacted with the same molecular complex, which dissociated under reducing conditions into polypeptides of M_r 90000 (minor), 57000, 47000 and 41000. MAb 44D10, 50B4 and 50C7 were also shown to recognize the same antigen on ALL cells, namely a glycoprotein of M_r 85000 (Quackenbush & Letarte, 1984). Thus for each of these three antigens, at least two MAbs can be tested with the various brain fractions.

Of the four antibodies initially tested, three were reactive with human brain homogenates (Table 2), 44D7 and 44H4 antibodies reacted with antigens in both grey and white matter. The level of 44D7 antigen in grey matter was approx. 4 times higher than in white matter, whereas the level of 44H4 in grey matter was approx. 2-fold that found in white matter. In contrast, the 44D10 antigen was found only in white matter. The 44H9 antigen was not detected in brain tissue, and thus MAb 44H9 was used as a negative control in subsequent experiments. Table 2 shows a 2-fold increase in 44D7 activity in the presence of Triton X-100, possibly due to an increase in the number of antigenic sites available for binding or to an activation of the antibody-antigen reaction. 44H4 and 44D10 antigenic activities were also higher in the presence of Triton X-100.

The specific activities of the antigens present in homogenates of white matter dissected from different regions of brain, including frontal, middle, occipital and mid-paraventricular were assayed after incubation with 1% Triton X-100 for 1 h. The 44H4 and 44D7 antigens were uniformly distributed among the white matter from the different regions studied. However, the 44D10 antigen, which was found only in white matter, was more concentrated in the occipital (7.2 units/mg of protein) and mid-paraventricular (5.7 units/mg of protein) than in the frontal and middle regions (4.2 units/mg of protein).

Distribution of antigens in subfractions of white matter

In order to identify the membrane structures expressing the ALL-brain antigens, the distribution of each antigen was determined in the subfractions obtained from human white matter (Scheme 1). The percentages of antigens recovered in the P₃ fraction compared with homogenate were 74, 66 and 54% for 44H4, 44D7 and 44D10 respectively, whereas only 5, 6 and 1% respectively were found in the purified myelin fraction (Table 3). Most of the remaining antigens were present in the S₁ fraction, which possessed light membrane fragments and soluble proteins. Further centrifugation of S₁ at higher centrifugal forces demonstrated that little, if any, of the 44D10 and 44D7

Fig. 1. Relative specific activity of 44H4, 44D7 and 44D10 antigens during fractionation of white matter

White matter was fractionated as shown in Scheme 1. All fractions were assayed for antigenic activity by inhibition of the appropriate c.r.i.a. Samples were incubated in 1% Triton X-100 for 1 h before assay. Specific activity is expressed in units of antigen/mg of protein for each fraction, and relative specific activity is calculated by assuming a value of 1.0 for that of the homogenate. ▨, myelin fraction, lowest inspecific antigenic activity; ■, P₃C fraction, highest in antigenic activity.

antigens were present in soluble form. When P₃, which contained the highest concentration of antigens, was fractionated further on a discontinuous gradient, fraction P₃C (1.0/1.2M-sucrose interface) was found to be enriched 3–6 times in 44H4, 44D7 and 44D10 antigens (Fig. 1). The enrichment is expressed in terms of specific activity (units of antigen/mg of protein) relative to that of the white-matter homogenate. In comparison, the myelin fraction isolated in the same experiment contained relative specific antigenic activities of only 0.1–0.6. These results would suggest that the three brain ALL antigens studied

Fig. 2. Electron micrographs of various subfractions obtained from human white matter

(a) Myelin fraction; (b) P₃ fraction; (c) P₃C fraction. Magnifications: $\times 8730$ (left) and $\times 87300$ (right).

are not localized in multilamellar, compact myelin, but in a heavier membrane fraction.

Characterization of the membrane fractions from white matter enriched in the antigens 44H4, 44D7 and 44D10

The sucrose-gradient fractions obtained from white matter and enriched in antigens, P₃ and P₃C were compared with compact myelin by electron microscopy and SDS/polyacrylamide-gel electrophoresis. In contrast with the purified myelin preparation, which consisted predominantly of compact, multilamellar myelin, the P₃ fraction contained several membrane structures, including myelin with fewer lamellae, small myelin fragments, mitochondria and other membranous structures (Fig. 2). The electron micrographs of the P₃C fraction revealed looser-packed myelin membrane structures and many smaller membrane fragments. The less compact myelin and the membrane fragments may be derived from structurally unstable

Fig. 3. Polypeptide composition of various fractions obtained from human white matter

An 80 μ g portion of protein from each fraction was electrophoresed on 12.5% (w/v) polyacrylamide gels and stained with Coomassie Blue. Lane 1, myelin; lane 2, P_3 fraction; lane 3, P_3C fraction. Abbreviations used: W, Wolfgram protein; L, lipophilin protein; BP, basic protein. The migration of the M_r markers is indicated on the right: phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000) and soya-bean trypsin inhibitor (20000).

myelin and from myelin internodes (Pereyra & Braun, 1983).

Membrane proteins solubilized from myelin, P_3 and P_3C were analysed on 12.5% (w/v) polyacrylamide gels (Fig. 3). The compact myelin fraction showed basic protein, lipophilin and the Wolfgram doublet present in the expected amounts (Boggs *et al.*, 1982). Although these proteins were also found in the P_3 and P_3C fractions, they were

Fig. 4. Immunoblot analysis of brain and ALL-cell antigens (a) Comparison of the 44H4 antigen from brain and ALL cell extracts. A 85 μ g portion of protein from extracts of white matter (lane 1) or HOON cells (lane 2) was electrophoresed on 10% (w/v)-polyacrylamide gels and transferred to nitrocellulose sheets. The blots were overlaid with 44H4 monoclonal antibody and then with 125 I-RAM-Fc as described in the Materials and methods section. (b) Reactivity of anti-MAG antibody with proteins from myelin and HOON-cell extracts. A 80 μ g portion of protein from either HOON-cell extract (lane 1), myelin (lane 2), or 10 μ g of soluble protein obtained after centrifugation of myelin preincubated at 37°C for 20 min (lane 3) were electrophoresed on 12.5% (w/v)-polyacrylamide gels and then transferred to nitrocellulose sheets. The blots were allowed to react with human anti-MAG IgM and then with 125 I-rabbit anti-human IgG.

decreased substantially, particularly lipophilin. A 50000- M_r band was prominent in both P_3 and P_3C and remains unidentified.

Identification of antigens associated with white matter by immunoblot analysis

To demonstrate that the antigenic determinants found in brain were present on molecules similar to those identified on ALL cells, proteins from extracts were separated by SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose

Fig. 5. Purification from a white-matter extract of the antigen recognized by MAb 44D7

Samples (100 µg) purified from an extract of white-matter P_3 fraction by affinity to 44D7-IgG-Sepharose 4B were reduced with dithiothreitol (lane 1) before alkylation or alkylated directly (lane 2) and separated by SDS/10% (w/v)-polyacrylamide-gel electrophoresis. M_r markers are indicated on the left. Samples and markers were stained with Coomassie Blue.

filters, and allowed to react with monoclonal antibodies. The 44H4 antigen in brain and ALL cells had an M_r of 94000 (Fig. 4a). The brain extract also contained a minor component of M_r 90000 absent from ALL cells. Whether this component represents a precursor or a degradation product of the main component remains to be established.

Because the M_r of 44H4 antigen was similar to that of myelin-associated glycoprotein (MAG), electroblots of brain and ALL-cell proteins were allowed to react with an antibody to MAG. White matter contained a reactive diffuse band that consisted of the 100000- and 90000- M_r species characteristic of MAG (Fig. 5). The latter is a degradation product of MAG and represents the soluble form of the molecule. No MAG-like antigen was detected in the ALL-cell protein extract. These results indicate that 44H4 antigen is not associated with MAG.

Identification of 44D7 and 44D10 antigens from white matter

Since MAb 44D7, 44H7, 50B4 and 50C7 were unreactive by the immunoblotting technique, the corresponding antigens were purified by immunoaffinity and identified by SDS/polyacrylamide-gel electrophoresis. Antigen 44D7 (44H7) was purified by MAb (either 44D7 or 44H7) affinity chromatography from a Triton X-100 extract of the P_3 subcellular fraction of brain white matter (Scheme 1). The enrichment in 44D7 antigen in the bound and eluted material relative to the P_3 fraction was of the order of 600-fold (average for three preparations). Since the P_3 fraction was enriched 3.5-fold in 44D7 antigen relative to the brain homogenate (Fig. 1), the final purification achieved was of the order of 2000-fold. Fig. 5 shows that the purified antigen, stained with Coomassie Blue, was seen as a diffuse band of $M_r > 120000$ under non-reducing conditions, in the presence of SDS. When the sample was reduced with dithiothreitol before electrophoresis, it was resolved into four polypeptide chains, of M_r 80000, 57000, 47000 and 41000. Minor components of smaller M_r are also visible. The M_r of the polypeptide subunits were similar to those observed for HOON leukaemic-cell line (Quackenbush & Letarte, 1985). When the purified 44D7 antigen was radiolabelled and immunoprecipitated with either 44D7 or 44H7 MAb, the gel pattern observed for the specifically immunoprecipitated antigens was similar to that of the Coomassie Blue-stained antigen shown in Fig. 5.

44D10 (50B4) antigen was purified from brain white-matter P_3 fraction by affinity to 50B4-IgG-Sepharose. The specifically bound and eluted material was radiolabelled and subjected to immunoprecipitation. Both 44D10 and 50B4 MAb precipitated a polypeptide chain of M_r 80000 under reducing conditions and 75000 under non-reducing conditions (Fig. 6). MAb 50C7 reactive with a different epitope of the same antigen on ALL cells also precipitated a similar molecule (Fig. 6). The M_r calculated for the antigen purified from brain white matter is smaller than that observed

Fig. 6. Immunoprecipitation of the 44D10 antigen from white matter

A 25 μ g sample of material purified from brain white matter or from a B-lymphoblastoid cell line (GM 3104) by affinity to the 50B4 immunoadsorbent was radiolabelled. Portions were incubated with saturating amounts of 50B4, 44D10, 50C7 or control MAb and immunoprecipitated as described in the Materials and methods section. The B-lymphoblastoid cell-line antigen was precipitated with MAb 50B4, and applied to lanes 1 and 2 of the SDS/10%-acrylamide gel. Portions of the antigen purified from white matter were precipitated with MAb 50B4 (lanes 3 and 4), 44D10 (lanes 5 and 6) and 50C7 (lanes 7 and 8) and were fractionated by SDS/polyacrylamide-gel electrophoresis along with the negative control (lane 9). Samples from lanes 1, 3, 5, 7 and 9 were reduced with dithiothreitol before alkylation with iodoacetamide.

for the antigen isolated from a B-lymphoblastoid-cell line extract by affinity to 50B4-IgG (Fig. 6). The M_r of the B-cell-line antigen was calculated as 85 000 and 80 000 under reducing and non-reducing conditions respectively.

Discussion

In the present study we have identified three proteins of human brain that share antigenic determinants with proteins found on a non-T, non-B ALL-cell line, HOON. The brain antigens were compared with the antigens of HOON cells by SDS/polyacrylamide-gel electrophoresis. A single polypeptide chain of M_r 94 000 was reactive with monoclonal antibodies 44H4 and 44E3 in both the ALL-cell line and brain white matter. An additional minor band of M_r 90 000 was seen on the

immunoblots of the white-matter samples, which may represent a proteolytically digested or an altered form of the major component. The 44H4 (44E3) antigen is similar in size to MAG, which has been shown recently to be reactive with HNK-1 antibody prepared against human natural killer cells (McGarry *et al.*, 1983). However, the lack of reactivity of anti-MAG antibody with ALL cells (Fig. 4) indicated that 44H4 antigen was not related to MAG nor to HNK-1 antigen.

A second antigen was identified in brain white matter by monoclonal antibodies 44D10, 50B4 and 50C7. The brain glycoprotein recognized by these antibodies has a M_r of 80 000, compared with 85 000 on a B-lymphoblastoid line (Fig. 5). Two other antigens, Thy-1 and F10-44-2, when isolated from brain, showed a lower M_r than the corresponding antigens isolated from lymphocytes (Barclay *et al.*, 1976; McKenzie *et al.*, 1982). These differences were demonstrated in the case of Thy-1 antigen to be due to differential glycosylation rather than to variations in the polypeptide-chain amino acid sequence (Kuchel *et al.*, 1978; Campbell *et al.*, 1981). Because of similarities in tissue distribution and M_r between the antigens recognized by 44D10 (50B4) and F10-44-2 antibodies, we have performed inhibition studies between our purified antigens and the F10-44-2 antibody (provided by Dr. J. Fabre). The antigens purified from either brain white matter or the leukaemic-cell line HOON by affinity to 50B4 IgG blocked completely the binding of F10-44-2 to leukaemic cells. These results indicate that the p80 glycoprotein antigen shared between ALL and brain white matter also carries the previously described F10-44-2 specificity (Dalchau *et al.*, 1980; McKenzie *et al.*, 1982).

The 44D7 and 44H7 antigens under non-reducing conditions, in the presence of SDS, appear as a molecular complex of M_r > 120 000 when purified from extracts of either white matter or HOON leukaemia cells. The antigen purified 2000-fold from brain white matter should be relatively pure on the basis of the observation that 1 unit of antigenic activity is obtained with 1 μ g of protein, corresponding to values obtained for the antigen purified from leukaemic cells (results not shown). The multiple subunits observed on reduction of the antigenic complexes purified from either brain or leukaemic cells are of similar M_r . However, the 80 000- M_r component, which forms the major band seen in the Coomassie Blue-stained gel, is not readily detected when the purified antigen is radiolabelled with 125 I, suggesting that tyrosine residues of that subunit might not be exposed in the intact high- M_r complex isolated by immunoaffinity. It is essential to establish if some of the lower- M_r subunits represent products of degradation of the higher- M_r subunits.

The distribution of 44H4, 44D7 and 44D10 antigens varies between grey and white matter of human brain. The 44H4 and 44D7 antigens are present in both grey and white matter; however, their concentration is 2- and 4-fold higher in grey than white matter respectively. In contrast, the 44D10 antigen is found only in white matter. Within white matter, all three antigens were found not to be associated with compact, multilamellar myelin, but rather with a denser membrane fraction consisting of loose myelin, myelin fragments and other membranes of unknown origin. We have recently shown that the 44D10 antigen is specifically associated with fibrous astrocytes (T. F. Cruz & E. J. Quackenbush, unpublished work) which are only found in white matter (Barr, 1979). Antigen F10-44-2, which was previously localized in white matter, was suggested to be associated with fibrous astrocytes (McKenzie *et al.*, 1982).

The monoclonal antibodies described in the current study have allowed us to identify antigens that do not represent major proteins of brain membrane fractions. Further studies on the localization in brain of these proteins, their possible association with certain cell types and the elucidation of their structure should provide insights into their biological roles.

This research was supported by grants from the Medical Research Council and from the National Cancer Institute of Canada. E. J. Q. is a recipient of a University of Toronto Connaught Studentship. T. F. C. is a Fellow of the Multiple Sclerosis Society of Canada. M. L. is a Research Associate of the National Cancer Institute of Canada. We thank Dr. J. Fabre for providing us with F10-44-2 antibody and Dr. P. Braun for supplying us with anti-MAG antibody.

References

- Barclay, A. N., Letarte-Muirhead, M. & Williams, A. F. (1975) *Biochem. J.* **151**, 699–706
- Barclay, A. N., Letarte-Muirhead, M., Williams, A. F. & Faulkes, R. A. (1976) *Nature (London)* **263**, 563–567
- Barr, M. L. (1979) *The Human Nervous System*, 3rd edn., Harper and Row, New York
- Boggs, J. M., Moscarello, M. A. & Papahadjopoulos, D. (1982) in *Lipid-Protein Interactions* (Jost, P. & Griffith, O. H.), vol. 2, pp. 1–51, Wiley and Sons, Chichester
- Campbell, D. G., Gagnon, J., Reid, K. B. M. & Williams, A. F. (1981) *Biochem. J.* **195**, 15–30
- Cotmore, S. F., Crowhurst, S. A. & Waterfield, M. D. (1981) *Eur. J. Immunol.* **11**, 597–603
- Dalchau, R. & Fabre, J. W. (1979) *J. Exp. Med.* **149**, 576–591
- Dalchau, R., Kirkley, J. & Fabre, J. W. (1980) *Eur. J. Immunol.* **10**, 745–749
- Kuchel, P. W., Campbell, D. G., Barclay, A. N. & Williams, A. F. (1978) *Biochem. J.* **169**, 411–417
- Letarte, M. & Falk, J. (1982) *J. Immunol.* **128**, 217–223
- Letarte-Muirhead, M., Barclay, A. N. & Williams, A. F. (1975) *Biochem. J.* **151**, 685–697
- Letarte, M., Teh, H.-S. & Meghji, G. (1980) *J. Immunol.* **125**, 370–377
- Letarte, M., Iturbe, S. & Quackenbush, E. J. (1984) *Mol. Immunol.* in the press
- Lowden, J. A., Moscarello, M. A. & Morecki, R. (1966) *Can. J. Biochem.* **44**, 567–577
- Maizel, J. V. (1971) *Methods Virol.* **5**, 179–246
- Markwell, M. A. K. & Fox, C. F. (1978) *Anal. Biochem.* **86**, 184–192
- McGarry, R. C., Helfand, S. L., Quarles, R. H. & Roder, J. C. (1983) *Nature (London)* **306**, 376–378
- McKenzie, J. L. & Fabre, J. W. (1981) *J. Immunol.* **126**, 843–850
- McKenzie, J. L., Dalchau, R. & Fabre, J. W. (1982) *J. Neurochem.* **39**, 1461–1466
- Okamura, J., Okano, H., Ikuno, Y., Tasaka, H., Motomura, S., Letarte, M. & Gelfand, E. W. (1984) *Leukaemia Res.* **8**, 97–104
- Pereyra, P. M. & Braun, P. E. (1983) *J. Neurochem.* **41**, 957–973
- Peterson, G. L. (1979) *Anal. Biochem.* **100**, 201–220
- Quackenbush, E. J. & Letarte, M. (1985) *J. Immunol.* in the press
- Reif, A. E. & Allen, J. M. V. (1964) *J. Exp. Med.* **120**, 413–433
- Schuller-Petrovic, S., Gebhart, W., Lassmann, H., Rumpold, H. & Kraft, D. (1983) *Nature (London)* **306**, 179–181
- Strandberg, R., McMaster, W. R., Sunderland, C. A. & Williams, A. F. (1978) *Eur. J. Immunol.* **8**, 832–839
- Swanstrom, R. & Shank, P. R. (1978) *Anal. Biochem.* **86**, 184–192
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Williams, A. F., Galfrè, G. & Milstein, C. (1977) *Cell* **12**, 663–673