Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4552–4556, July 1987
Immunology

Cloning of a brain protein identified by autoantibodies from a patient with paraneoplastic cerebellar degeneration*

(paraneoplastic syndrome/cDNA expression libraries/autoimmunity)

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Contributed by Lloyd J. Old, February 13, 1987

ABSTRACT **Autoantibodies directed against neuronal proteins have been identified in some patients with paraneoplastic cerebellar degeneration. To identify the molecular targets for these autoantibodies, we constructed a λgt11 cDNA expression library from human cerebellum and screened the library with IgG from a patient with paraneoplastic cerebellar degeneration. A single clone, pCDR2, produced a fusion protein that reacted strongly with the patient's IgG. The isolated pCDR2 clone was used to identify six overlapping cDNA clones. Sequencing of the pCDR clones revealed a distinctive pattern consisting of a unit of 18 nucleotides (6 amino acids) repeated in tandem along the entire cDNA sequence. This sequence is unlike any previously described eukaryotic gene. Southern blot analysis was consistent with single-copy representation of the CDR (cerebellar degeneration-related) gene in the human and mouse genome. RNA blotting studies with normal tissues showed expression of the CDR gene to be largely restricted to brain. Expression of the CDR message was also noted in cell lines derived from cancers of neuroectodermal, kidney, and lung origin.**

Several distinctive neurologic disorders of unknown etiology occur as "paraneoplastic syndromes" or "remote effects" of systemic cancer (1). The syndrome of paraneoplastic cerebellar degeneration (PCD) is among the most common of these disorders and generally occurs in patients with neoplasms of the lung (especially small cell carcinoma), breast, ovary, or with Hodgkin disease (1, 2). Neuropathologic features of PCD include extensive loss of Purkinje cells, degenerative changes in the remaining Purkinje cells, variable loss of granule and basket neurons, proliferation of Bergmann glial cells, and a variable degree of perivascular and meningeal infiltration by lymphocytes and other mononuclear cells. The presence of anti-Purkinje cell antibodies in some PCD patients suggests an autoimmune etiology (3–6). Immunofluorescent staining of Purkinje cell cytoplasm has been reported in cases of PCD associated with Hodgkin disease (3), small cell lung cancer (4), breast cancer (5), and ovarian cancer (5, 6). Several investigators have attempted to characterize the putative neuronal antigens reacting with anti-Purkinje cell antibodies. Immunoblots with sera from PCD patients have identified several brain proteins of various molecular weights (7–10), but the identity of these "target" proteins and their role in the pathogenesis of PCD remain unknown. Direct efforts at purifying the target proteins have been hampered by their restricted cellular distribution and relatively low abundance. We have used the autoantibodies from a patient with PCD to identify and clone a gene from a human cerebellum cDNA library. The gene is expressed predominantly in neuroectodermal tissues and encodes a protein with a distinctive tandemly repeating structure.

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MATERIALS AND METHODS

Human and Mouse Tissue. Normal human cerebellar cortex, cerebral cortex, and other tissues were obtained at autopsy. BALB/c mice were from our breeding colony. Tumor cell lines were from the Sloan–Kettering Institute collection.

DNA and RNA Preparations. High molecular weight cellular DNA and total cellular RNA were prepared as described (11). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose column chromatography (12).

Construction of λgt11 cDNA Library. A λgt11 cDNA library was constructed from human cerebellar poly(A)$^+$ RNA according to Huynh *et al.* (13). cDNA species >300 base pairs (bp) were size-selected by 5% polyacrylamide gel electrophoresis and ligated to *Eco*RI-digested dephosphorylated λgt11 arms in a molar ratio of 2:1 (13, 14). The ligated DNA was packaged using a high-efficiency *in vitro* packaging extract (Vector Cloning Systems, San Diego, CA). Approximately 2.3×10^5 plaques were obtained, with 70% being recombinants. An amplified stock of the library was prepared in *Escherichia coli* Y1088 (12) and used for antibody screening.

Antibody Screening of λgt11 Library. Serum used for screening was obtained from a patient with adenocarcinoma of unknown origin, PCD, and high-titer anti-Purkinje cell antibodies. IgG was isolated from the serum by ammonium sulfate precipitation and DEAE-Sephadex chromatography (15). Recombinant phage were screened at a density of 8000 plaque-forming units per 85-mm plate of *E. coli* Y1090. The plates were incubated at 42°C for 4 hr, overlaid with nitrocellulose filters (Millipore) saturated with isopropyl β-D-thiogalactopyranoside (IPTG), and incubated 3 hr at 37°C. Filters were washed in Tris·HCl/NaCl/Tween 20 and incubated at 4°C overnight with the primary antibody solution containing 2% bovine serum albumin and preabsorbed with a lysate of *E. coli*. Incubation at room temperature for 4 hr with horseradish peroxidase-conjugated goat anti-human IgG (Bio-Rad) was followed by staining with chloronaphthol (Bio-Rad). Clones yielding positive signals were purified by several rounds of antibody screening until 100% of the plaques gave positive signals.

Preparation and Immunoblotting of λgt11 Fusion Proteins. Lysogens of recombinant phage were prepared in *E. coli* Y1089 (13) at a multiplicity of infection of 5. Cultures were grown at 32°C to an OD_{550} of 0.5, followed by induction of lytic growth at 44°C for 30 min, addition of IPTG to a concentration of 10 mM, and incubation at 37°C for 2 hr.

Abbreviations: PCD, paraneoplastic cerebellar degeneration; IPTG, isopropyl β-D-thiogalactopyranoside; CDR, cerebellar degeneration-related.

*A portion of this work was presented at the 38th annual meeting of the American Academy of Neurology, New Orleans, May 1, 1986.

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Bacterial pellets were lysed in cold Tris·HCl/EDTA/lysozyme buffer (16), sonicated, and centrifuged. The supernatants were diluted in $NaDodSO_4$/polyacrylamide gel sample buffer containing 5% 2-mercaptoethanol, electrophoresed in 7% $NaDodSO_4$/polyacrylamide gel (17), and electrophoretically transferred to nitrocellulose (18). Blots were analyzed with the patient's serum as described above for antibody screening.

Plaque Hybridization. Nitrocellulose filters were overlaid on 85-mm phage plates for 4 min, washed at room temperature for 4 min each in 1 M NaCl/0.5 M NaOH, 2 M NaCl/1 M Tris·HCl, pH 6.8, and 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), then vacuum-dried at 80°C for 2 hr. Filters were hybridized and washed as described for Southern blot analysis (see below), except that dextran sulfate was omitted from the hybridization solution.

DNA Sequencing and Analysis. cDNA clones were subcloned into M13mp18 and M13mp19, sequenced by the dideoxy chain-termination method (19), and analyzed with computer programs from BIONET.

RNA Blot and Cytoplasmic Dot Blot. RNA blot and RNA dot blot analyses were done as described (20) using ^{32}P-labeled nick-translated probes. For cytoplasmic dot blot analysis, cultured tumor cells were washed with phosphate-buffered saline, lysed in Tris·HCl/EDTA/0.5% Nonidet P-40 containing 0.1 mM vanadyl ribonucleoside complex (Bethesda Research Laboratories), and centrifuged (21). The supernatants were transferred to an equal volume of 12× SSC and 15% formaldehyde, bound to nitrocellulose paper in a Minifold apparatus (Schleicher & Schuell), and analyzed with nick-translated probes.

Southern Blot Analysis. High molecular weight genomic DNA (15 μg) was digested with restriction endonucleases, electrophoresed in 0.7% agarose gels, and transferred to nitrocellulose (22). Filters were prehybridized and hybridized to nick-translated probes at 65°C for 15 hr. Hybridization was performed in 5× SSC/4× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/50 mM phosphate buffer/50 μg of salmon sperm DNA per ml/10% (vol/vol) dextran sulfate. Filters were then washed and autoradiographed.

RESULTS

Isolation of Positive Clones. Initial antibody screening of 110,000 λgt11 recombinants from the human cerebellar cDNA library by the PCD patient's IgG yielded a single reproducibly positive clone designated pCDR2. To confirm reactivity with antigenic determinants encoded by the cDNA fusion protein, pCDR2 and wild-type λgt11 were used to infect lysogenic strain Y1089. Bacterial lysates were prepared after induction of the phage β-galactosidase gene by IPTG, and analyzed by $NaDodSO_4$/PAGE (Fig. 1). The 116-kDa β-galactosidase present in the wild-type culture was replaced by a 140-kDa fusion protein in the pCDR2 culture. Immunoblotting revealed that this fusion protein reacted specifically with the patient's IgG. No reaction was seen with IgG from normal individuals.

The pCDR2 clone was found to contain a 320-bp cDNA insert, which was then used as a probe to rescreen the cerebellar cDNA library. Six positive overlapping clones were obtained (designated pCDR5, -6, -8, -12, -13, and -18) with inserts ranging in size from 500 to 1200 bp. None of the six clones identified by plaque hybridization produced a fusion protein that reacted with the patient's IgG. The pCDR13 clone was studied in detail, as it was the largest clone and contained overlapping sequences of the other six clones.

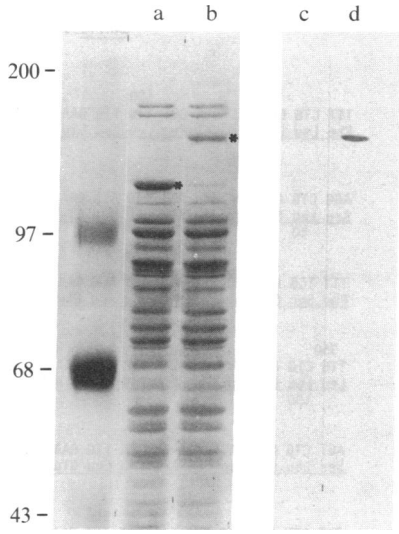

FIG. 1. Characterization of the pCDR2 fusion protein. Lanes a and b, extracts of IPTG-induced lysogens from wild-type λgt11 and pCDR2, respectively, were electrophoresed in 7% $NaDodSO_4$/polyacrylamide gel, and stained with Coomassie blue. Bands at 116 and 140 kDa in lanes a and b (asterisks) represent β-galactosidase and the pCDR2 fusion protein, respectively. Lanes c and d, immunoblots of extracts from lanes a and b with the PCD patient's IgG, as visualized with the peroxidase technique. Molecular mass markers (in kDa) are indicated on the left.

Sequence Analysis and Repetitive Features of Cerebellar Degeneration-Related (CDR) Clones. Fig. 2 shows the restriction map of pCDR13 and the six other clones. Fig. 3 shows the nucleotide and derived amino acid sequences of pCDR13. A continuous open reading frame commences with an ATG codon at position 55. This is the only long open reading frame present in the sequence, and it is in register with the junctional lacZ sequence of λgt11. The 669-nucleotide open reading frame is followed by a 3′ untranslated sequence. The protein encoded by the sequence from nucleotide positions 55–724 is composed of 223 amino acids and has a calculated molecular mass of 27,038 Da.

The most striking feature of the sequence is a pattern of tandem repeats consisting of units of 18 nucleotides throughout the entire cDNA sequence. The derived amino acid sequence contains 34 inexact tandem repeats of six amino acids that compose 91% of the predicted peptide (Figs. 3 and 4). The hexapeptide repeating unit features a nearly invariant core of Glu-Asp (codons GAA GAC), flanked on the amino side by hydrophobic residues (generally leucine and phenyl-

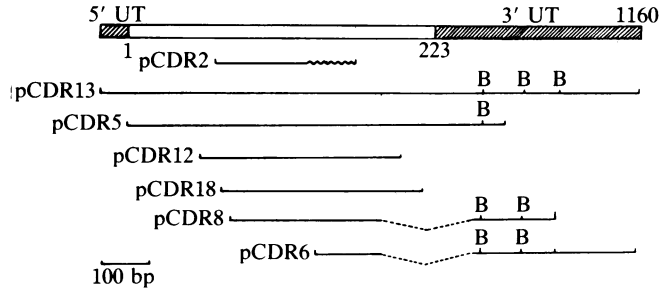

FIG. 2. Restriction map of the CDR cDNA clones. Seven clones were aligned by DNA sequencing and by comparison of *Bgl* II restriction sites (*B*). Clones pCDR6 and pCDR8 lacked segments presented in pCDR13 (dashed lines). pCDR2 contained a 120-bp sequence in its 3′ end (wavy line) that was not present in the other clones and was proved to be a cloning artifact. Hatched areas in the composite sequence (top) represent 5′ and 3′ untranslated regions (UT) of pCDR13.

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                         1                                                 50
                         GAATTCCGAGCTTAGTTGGAAGACATATATTTTTGGAAGACGTGGATTTTCTGGAAGAC ATG GCT TGG TTG GAA GAC GTG GAT
                                                                                      MET Ala Trp Leu Glu Asp Val Asp
                                                                                       1

                            100                                                   150
TTT CTG GAA GAC GTA CCT TTG TTG GAA GAC ATA CCT TTG TTG GAA GAC GTA CCT TTG TTG GAA GAC GTA CCT TTG TTG GAA GAC ACA AGT
Phe Leu Glu Asp Val Pro Leu Leu Glu Asp Ile Pro Leu Leu Glu Asp Val Pro Leu Leu Glu Asp Val Pro Leu Leu Glu Asp Thr Ser
                                             20

                                      200                                                                 250
AGG CTG GAA GAC ATT AAT TTG ATG GAA GAC ATG GCT TTG TTG GAA GAC GTG GAT TTG CTG GAA GAC ACG GAT TTC CTG GAA GAC CTG GAT
Arg Leu Glu Asp Ile Asn Leu Met Glu Asp Met Ala Leu Leu Glu Asp Val Asp Leu Leu Glu Asp Thr Asp Phe Leu Glu Asp Leu Asp
 40                                                                          60

                                                    300
TTT TCG GAA GCT ATG GAT TTG AGG GAA GAC AAG GAT TTT CTG GAA GAC ATG GAT AGT CTG GAA GAC ATG GCT TTG TTG GAA GAC GTG GAC
Phe Ser Glu Ala Met Asp Leu Arg Glu Asp Lys Asp Phe Leu Glu Asp Met Asp Ser Leu Glu Asp Met Ala Leu Leu Glu Asp Val Asp
                                             80

350                                                               400
TTG CTG GAA GAC ACG GAT TTC CTG GAA GAC CCG GAT TTT TTG GAA GCT ATA GAT TTA AGG GAA GAC AAG GAT TTT CTG GAA GAC ATG GAT
Leu Leu Glu Asp Thr Asp Phe Leu Glu Asp Pro Asp Phe Leu Glu Ala Ile Asp Leu Arg Glu Asp Lys Asp Phe Leu Glu Asp Met Asp
 100                                                                         120

               450                                                            500
AGT CTG GAA GAC CTG AGG CCA TTG GAA GAT GTG GAT TTT CTG GAA GAC ATG GCT TTT TTG GAA GAC GTA GAT TTT CAG GAA GAC CCA AAT
Ser Leu Glu Asp Leu Arg Pro Leu Glu Asp Val Asp Phe Leu Glu Asp Met Ala Phe Leu Glu Asp Val Asp Phe Gln Glu Asp Pro Asn
                                            140

                          550                                                     600
TAT CCG GAA GAC TTG GAT TGT TGG GAA GAC GTG GAT TTT CTG GAA GAC TGG AGG TTA CTG GAA GAC ATG GAT TTT CTG GAA GAC ATG GAT
Tyr Pro Glu Asp Leu Asp Cys Trp Glu Asp Val Asp Phe Leu Glu Asp Trp Arg Leu Leu Glu Asp Met Asp Phe Leu Glu Asp Met Asp
 160                                                                         180

                                     650                                                                  700
TTT CTG GAA GAC GTG GAT CTT CAG GAA GAC ATA TAT TGG CTG GAA GAC CTG GAT TTT TTC CGG AAG ATG TGG ATT GAC TGG AAG ACC TGG
Phe Leu Glu Asp Val Asp Leu Gln Glu Asp Ile Tyr Trp Leu Glu Asp Leu Asp Phe Phe Arg Lys Met Trp Ile Asp Trp Lys Thr Trp
                                            200

                                          750                                                 800
ATT TGG TGG AAG ACG TAG ATTTTTCTGGAAGACACTGACTGACTGGAAGACCTGGATTTCTTTCTGGAAGACACTGATTGACTGGAAGACCTGGATTTCTTTCTGGAAGACAC
Ile Trp Trp Lys Thr ***
 220

                          850                                                 900
TGATTGACTGGAAGATCTAGATTTTTCTGGAAGAACTAGATTTACTGGAAGACTTGGATTTGGTGGAAGACACAGATTTTTCTGGAAGACATGGATTAGCTGGAAGATCTGTATTTGAT

            950                                     1000                                                 1050
GGAAGACCTTGAAATTATTGGAAGACATGGATTTCCTGGAAGACGTGGATTTTCCTGGAAGATCTGGATTTGGTGGAAGACCAGTAATTGCTGGAAGACTGGATTTGCTGGAAGACTTG

                                   1100                                                 1150
ATTTACTGGAAGACTTGGAGCTTCTTGGAAGACATGGATTGTCCGGAAGACATGGATTGTCTGGAAGATGTGGATTTTCTGGAAGCTCAGGATTATCTGG
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FIG. 3. Nucleotide sequence and derived amino acid sequence of pCDR13 cDNA. The *Eco*RI linker sequence is shown prior to nucleotide position 1. The numbers above and below the sequences are nucleotide and amino acid positions, respectively. The 18-nucleotide tandem repeating units are underlined. Asterisks indicate the translational termination codon.

alanine) and on the carboxyl side by an aliphatic residue, usually followed by aspartic acid. A nonrepetitive segment of 17 amino acids is found at the carboxyl terminus of the peptide due to a single nucleotide insertion that disrupts the reading frame.

A hydropathy profile (23) of the predicted amino acid sequence (Fig. 5) reveals an initial hydrophobic segment, followed by cycles of alternating very hydrophilic and slightly hydrophobic regions with a periodicity of ≈40 amino acid residues, and ending with a slightly hydrophilic nonrepetitive segment. The predicted secondary structure of the sequence according to the method of Chou and Fasman (24) is an α-helix, with a β-sheet conformation extending along 30 amino acids at the carboxyl end.

In comparison to the pCDR13 sequence, pCDR6 and pCDR8 both lack a 204-bp segment that includes the translational termination codon of pCDR13. This indicates a possible alternative splicing of mRNA, as supported by the presence of multiple mRNA species in RNA blots (see below). Because of the deletion, the predicted amino acid sequences of pCDR6 and pCDR8 are slightly different from

Nucleotide:	TT(G/T)	(C/T)TG	GAA	GAC	(A/G)TG	GAT
Amino acid:	Leu/Phe	Leu	Glu	Asp	Met/Val	Asp
No. appeared:	26	26	34	32	18	21

FIG. 4. Tandem repeats in the CDR sequence. The sequence of the 34 repeats is summarized to illustrate the consensus 18-nucleotide/hexapeptide unit.

pCDR13 in the carboxyl end of the molecule, with the main repetitive feature remaining unchanged.

Southern Blot Analysis of the CDR Gene in Human and Mouse. Southern blot analysis showed hybridization of the 1.2-kilobase (kb) pCDR13 probe to single fragments in digests of human genomic DNA by *Bam*HI, *Eco*RI, *Hin*dIII, *Pst* I, and *Pvu* II (Fig. 6). These findings are consistent with single-copy representation of the CDR gene. The observation that different restriction enzymes generated single fragments also suggests that the entire pCDR13 cDNA clone was derived from one exon or from exons lacking restriction sites in the intervening sequences. Southern analysis of restriction digests of mouse genomic DNA also showed strong hybrid-

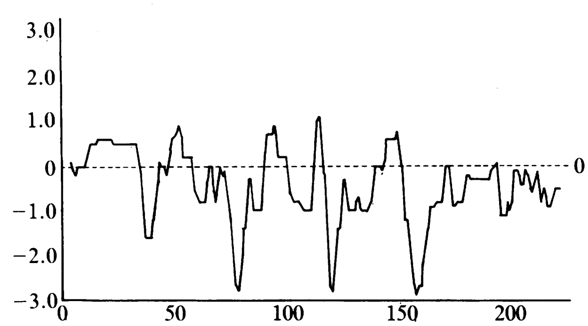

FIG. 5. Hydropathy plot of the predicted amino acid sequence of the CDR protein based on the method of Kyte and Doolittle (23). Hydropathy values for a span of seven amino acid residues were averaged, assigned to the middle residue of the span, and plotted (*y* axis) according to the position of the middle residue along the sequences (*x* axis).

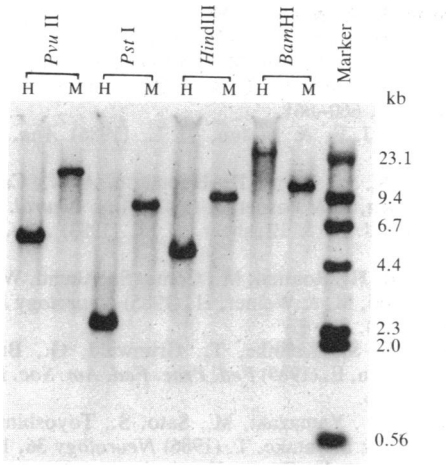

FIG. 6. Southern blot analysis of genomic DNA fragments. Total cellular DNA was prepared from human (lanes H) and mouse (lanes M). DNA (15 μg per lane) was digested with restriction enzyme, electrophoresed through a 0.7% agarose gel, transferred to a nitrocellulose filter, and hybridized with ^{32}P-labeled pCDR13 probe.

ization signals (Fig. 6), indicating that the CDR gene is highly conserved between human and mouse.

Analysis of CDR Transcripts in Normal and Tumor Cells. Fig. 7 shows the RNA dot blot analysis of CDR transcripts in human and mouse tissue. CDR mRNA was detected in abundant amounts in human cerebellar cortex and cerebral hemisphere cortex, but it was only barely detectable in lung, kidney, and heart muscle. At least 500-fold differences were estimated between the levels of CDR messages in brain and non-neural tissues. In mouse tissue, the mRNA was estimated to be 5–10 times more abundant in cerebellum than in cerebrum and was undetectable in lung, heart, kidney, liver, and thymus.

Human tumor cell lines were also tested by cytoplasmic dot hybridization with the pCDR13 probe (Table 1). The message was present in all 10 neuroblastoma lines tested, as well as in the majority of renal cell carcinoma lines and a proportion of astrocytoma, melanoma, and lung carcinoma lines. The degree of expression by positive cell lines varied widely for a given tumor type, with no obvious correlation with morphological characteristics. The strongest expression was observed in several of the neuroblastoma and renal carcinoma lines, nearly approaching the abundance of the CDR message in normal brain tissue. The CDR message was

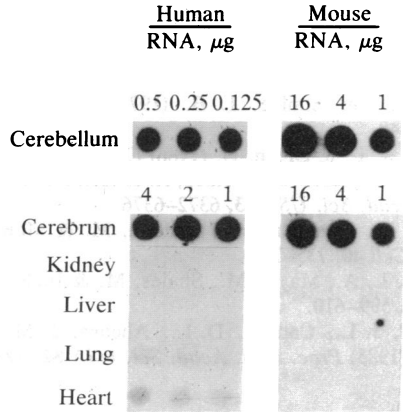

FIG. 7. Dot blot hybridization of total cellular RNA from human and mouse tissues with pCDR13. Total cellular RNA was prepared from postmortem human tissues and BALB/c mouse tissues, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled nick-translated pCDR13.

Table 1. Detection of CDR mRNA in human tumor cell lines by cytoplasmic dot blot analysis

Tumor cell lines	No. positive/ no. tested	% positive
Neuroblastoma	10/10	100
Renal cell carcinoma	8/12	67
Astrocytoma	7/14	50
Lung carcinoma	4/10	40
Melanoma	3/14	21
Lymphoma/leukemia	0/9	0
Colon carcinoma	0/10	0
Breast carcinoma	0/8	0
Other carcinomas*	0/6	0

*Two ovarian, one cervical, one bladder, one pancreatic, one choriocarcinoma.

not expressed in leukemia/lymphoma cell lines or in lines derived from colon or breast carcinoma.

RNA blots of CDR mRNA-positive human cell lines demonstrated three closely migrating mRNA species (from 1.3 kb to 1.5 kb), best illustrated by the neuroblastoma line SMS-KAN (Fig. 8). This mRNA heterogeneity may be due to alternative splicing, as suggested by structural studies of cDNA clones pCDR6 and pCDR8 (Fig. 3). RNA blot analysis of rabbit and mouse brain confirmed the greater expression of the CDR gene in cerebellum than in cerebrum (Fig. 8). Rabbit CDR messages were similar in size to the human transcripts, except for the absence of the 1.3-kb species. In contrast, the blot of mouse brain RNA showed only a single 3.5-kb message.

DISCUSSION

To identify the molecular targets for anti-brain antibodies in PCD, we have used the serum from a patient with PCD to isolate a cDNA clone from a human cerebellum λgt11 expression library. The nucleotide sequence of the CDR cDNA contains a pattern of tandem repeats with a structure that is unlike any known eukaryotic gene. In addition, the

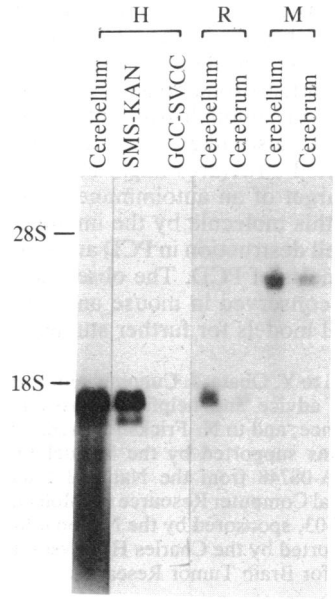

FIG. 8. RNA blot hybridization of ^{32}P-labeled pCDR13 with RNA from human (H), rabbit (R), and mouse (M) brain, neuroblastoma cell line SMS-KAN, and choriocarcinoma cell line GCC-SVCC. Amounts of RNA loaded were as follows: 5 μg of total RNA from brain, 5 μg of poly(A)$^+$ RNA from SMS-KAN and GCC-SVCC. Positions of 28S and 18S ribosomal RNAs are indicated.

pattern of expression of the CDR gene does not resemble any previously described neuroectodermal gene. CDR gene transcripts are found much more abundantly in normal adult brain, especially cerebellum, than in non-neural tissues. The low level of CDR message in human lung, kidney, and heart muscle could be due to expression in the small subpopulation of cells of neuroectodermal origin in these tissues.

Tandemly repeated sequences are known to characterize a number of surface proteins of malarial parasites. For example, the circumsporozoite protein of *Plasmodium falciparum* consists almost entirely of tandem repeats of 4 amino acids (25), while that of *Plasmodium knowlesi* contains 12 repeats of a 12-amino acid unit (26). These *Plasmodium* circumsporozoite proteins show a high degree of antigenicity and are able to serve as the targets for neutralizing antibodies. Most eukaryotic proteins with known tandem repeats are structural proteins, such as the intermediate filaments (27), nuclear lamins (28), myosin (29), and α-tropomyosin (30). These proteins form coiled coils and share a common structural pattern of tandemly repeating units of 7 amino acids, with hydrophobic residues occupying the first and fourth positions. However, there are otherwise no restrictions on the amino acids in the repeating units of these proteins, in contrast to the CDR molecule. Two structural proteins show certain similarities to the CDR gene product. Involucrin, a keratinocyte envelope component, contains a central segment of 39 repeats of 10 amino acids and appears to be a substrate for cross-linking by transglutaminase (31). The PrP prion protein, thought to polymerize into fibrils, contains well-conserved tandem repeats of 8 amino acids extending over 13% of its sequence (32, 33). To our knowledge, the only nonstructural eukaryotic protein with tandem repeats is RNA polymerase II, with a highly conserved unit of 7 amino acids repeated 26 times in the COOH-terminal sequence (34, 35). Knowledge of the tandem repeat composition of the CDR protein itself, therefore, does not allow insight into possible cellular functions.

Is the protein product of the CDR gene an important target in the pathogenesis of PCD? The fact that this molecule is recognized by the PCD patient's serum and that transcripts for it are expressed predominantly in neural tissues (especially cerebellum) is consistent with a significant disease association. The absence of the CDR mRNA in breast and ovarian cancer cell lines and its presence in primary tumors from two PCD patients (one breast adenocarcinoma and one ovarian carcinoma; unpublished data) add to a possible association. It is also likely that a molecule with such tandemly repeated structures might be highly immunogenic and thus the target of an autoimmune response. However, recognition of this molecule by the immune system may be secondary to cell destruction in PCD and not causally related to the pathogenesis of PCD. The observation that the CDR gene is highly conserved in mouse and rabbit may provide valuable animal models for further studies of this molecule.

We are grateful to Y. Obata, J. Cunningham, P. K. Srivastava, and J. Whitaker for advice and helpful discussion; to N. Golet for technical assistance; and to N. Fricks for preparing the manuscript. This research was supported by the Samuel Freeman Charitable Trust, Grant CA-08748 from the National Cancer Institute, and BIONET National Computer Resource for Molecular Biology, Grant 1 U41 RR-01685-03, sponsored by the National Institutes of Health. E.J.D. was supported by the Charles H. Revson Foundation and by the Association for Brain Tumor Research.

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