Proteins from Human Cerebrospinal Fluid: Binding with Nucleic Acids

Abstract. *Cerebrospinal fluid (CSF) contains two groups of proteins that bind tightly to DNA and to polyriboguanylic acid, respectively. In certain diseases the amounts of a given nucleic acid bound by a constant volume of CSF may increase, while in others the amount of such proteins may be reduced. Binding of polyriboguanylic acid increased in CSF samples from patients with brain tumors, stroke, multiple sclerosis, and communicating hydrocephalus, but it significantly decreased in CSF samples from patients with obstructive hydrocephalus. These increases may or may not be proportional to the rise in total CSF proteins characteristic for these diseases. Elevated binding of DNA was observed in samples from patients with hydrocephalus, epilepsy, and cortical atrophy. The technique described may be applicable to the diagnosis of a variety of diseases of the central nervous system.*

Nucleic acids carry information necessary for cellular growth and differentiation. These functions are accomplished in cooperation with a variety of proteins, and interactions between these macromolecules are of fundamental biochemical importance. Serum and ascites, amniotic, and cerebrospinal fluids contain variable amounts of proteins capable of reacting with DNA, with a synthetic polymer of riboguanylic acid [poly(rG)], and with a copolymer of guanylic and cytidylic acid residues (*1*). Only the first two nucleic acids [DNA and poly(rG)] were found to react with samples of human cerebrospinal fluid (CSF). The reactive molecules are being characterized. The proteins are of the same apparent size as those in serum (*1*); that is, the poly(rG)-binding activity appears together with gamma globulins in fractions eluted from columns of Sephadex G200, while the DNA-binding proteins are excluded from the same gel.

We now describe apparent correlations between cerebral pathology and concentrations of such proteins in CSF.

Binding of nucleic acids to CSF proteins was assayed by a modification (*2*) of the membrane filtration technique (*3*). Nucleic acids labeled (*4*) with radioactive (^3H or ^{32}P) precursors were mixed with CSF samples and placed in a water bath at 40°C. At various times samples were withdrawn and diluted into a detergent-containing salt solution (0.1 percent sodium dodecyl sulfate in 0.1*M* NaCl). This detergent dissolves complexes between nucleic acids and proteins, except for those formed via strong linkages between the two macromolecules. Subsequent filtration through the nitrocellulose membranes resulted in the retention of those nucleic acids that formed complexes with proteins, whereas naked native nucleic acids passed through the nitrocellulose filter. Thus, the amount of radioactivity associated with and detected on the filters reflects the degree of complex formation between nucleic acids and CSF proteins and, indirectly, the amounts of reactive proteins in the sample.

The amounts of nucleic acids complexed with proteins increased for the first 20 to 30 minutes, then leveled off (Fig. 1). Binding characteristics for the two CSF samples tested were completely different. Poly(rG) associated relatively strongly with CSF from patient A (a 57-year-old woman with brain tumor), while DNA interacted preferentially with that of patient B (a 2-year-old child with hydrocephalus).

The values for apparently normal subjects (that is, those without any appreciable pathology of the central nervous system) are 2 to 5 μg of poly(rG) and 0.5 to 2 ng of DNA bound per 1 ml of CSF at the end of the 60-minute incubation period (Table 1). Binding of poly(rG) was considerably increased in either primary or metastatic brain tumors, while no such effect was observed in patients with peripheral malignancies without brain metastases. A similar increase in the amounts of poly(rG) bound was observed in CSF samples from patients with stroke and multiple sclerosis. These increases were statistically highly significant except for astrocytomas, where the probability of error was between 0.01 and 0.05 because of the low number of cases. Samples of CSF from hydrocephalic patients fell into two distinct groups: one with unusually low and another with moderately high abilities to bind poly(rG). To the first group belong cases with obstructive, and to the second those with communicating, hydrocephalus.

Generally these diseases are characterized by moderate to high concentrations of CSF proteins. We therefore examined the relationship between the ability to bind poly(rG) and the protein content of a particular sample

Table 1. Binding of nucleic acids to CSF proteins at the end of 60 minutes of incubation at 40°C (plus or minus standard deviation). Numbers in parentheses indicate the number of cases in each category. At least three independent assays were made for each sample of cerebrospinal fluid.

Status of subjects	Poly(rG) binding		DNA (ng/ml CSF)
	CSF (μg/ml)	CSF protein (μg/mg)	
Normal donors (16)	3.3 ± 1.4	11 ± 2	1.1 ± 0.4
Carcinoma with metastases to brain (9)	21.1 ± 12.3*	16 ± 9	1.9 ± 0.3
Meningioma (6)	36.4 ± 17.0*	41 ± 23*	1.7 ± 0.3
Acoustic neuroma (9)	21.4 ± 11.1*	24 ± 11*	2.1 ± 0.4
Astrocytoma (4)	11.9 ± 6.8*	11 ± 4	
Other brain tumors (12)	10.8 ± 3.2*	14 ± 6	0.8 ± 0.6
Carcinoma, peripheral, no brain metastases (4)	4.4 ± 1.6	8 ± 4	0.9 ± 0.4
Stroke (21)	12.6 ± 4.2*	28 ± 10*	2.0 ± 0.8
Acute brain injury (8)	5.0 ± 2.1	9 ± 4	1.4 ± 1.0
Multiple sclerosis (8)	7.3 ± 2.7*	17 ± 7	2.0 ± 0.6
Hydrocephalus (ventricular fluid)	4.2 ± 3.0	6 ± 2†	2.1 ± 0.9
Obstructive (9)	0.8 ± 0.3†	3 ± 1†	
Communicating (11)	9.2 ± 4.7*	8 ± 3	
Hydrocephalus (lumbar fluid)	2.1 ± 1.4	11 ± 9	6.6 ± 2.8*
Obstructive (22)	0.7 ± 0.2†	3 ± 1†	
Communicating (18)	6.1 ± 1.9*	23 ± 10*	
Schizophrenia (3)	4.3 ± 1.8	11 ± 4	6.3 ± 4.1*
Alcoholism, chronic (4)	5.0 ± 2.0	13 ± 5	4.5 ± 1.1*
Cortical atrophy (27)	4.8 ± 1.2	14 ± 4	5.4 ± 2.4*
Epilepsy (35)	3.7 ± 2.0	11 ± 6	4.4 ± 1.9*
Headaches of unknown etiology, hysteria (14)	4.4 ± 2.8	12 ± 6	4.0 ± 2.8*

* Values are above normal. † Values are below normal.

(Table 1). Between 8 and 15 μg of poly(rG) were bound per milligram of CSF protein in samples from normal subjects. In certain diseases, notably stroke, meningioma, acoustic neuroma, and communicating hydrocephalus, this proportion was increased. No such increase was apparent in metastatic carcinoma, astrocytoma, or other brain tumors. We do not know whether these observations are of any potential significance for the diagnosis of brain malignancies.

Interaction with DNA was normal with CSF from patients with brain tumors and stroke; if anything, the binding apparently decreased in some patients with brain tumors. In separate experiments we found that this apparent decrease was not due to an increased activity of deoxyribonucleases in these CSF samples. In contrast, samples from patients with hydrocephalus, schizophrenia, alcoholism, cortical atrophy, seizures, or headaches of unknown etiology bound more DNA than those from normal subjects. For hydrocephalus and for the last four groups the increase was statistically highly significant. In all these groups there was a substantial and uniform increase in the amounts of DNA bound per milligram of CSF proteins (computations not shown in Table 1), since in general these diseases are not characterized by increases in total CSF proteins.

We collected a significant number of samples of ventricular fluid only from hydrocephalic patients. With few exceptions, the results were similar to those with lumbar fluid, if the relatively lower protein concentration in the ventricular fluid is taken into account.

The reproducibility of our data was satisfactory within a particular batch of nucleic acids. However, appreciable variability was noted when different lots of poly(rG) were used. The relatively high values for standard deviation were in part due to such variabilities. Obviously, further standardization of this indicator polymer is necessary for successful application of this method for clinical diagnosis.

We did not systematically study the effects of pharmacological and surgical treatments on nucleic acid–binding proteins in CSF (in more than 80 percent of cases only one sample of the fluid was obtained). Nevertheless, our initial observations indicate that such treatments can alter the concentrations of such proteins. Quantitative variations were also seen as the disease progressed. These factors further increased the standard deviation calculated.

The physiological role of the CSF proteins that react with nucleic acids and the reasons for their specificity are unknown at present.

We have suggested (*1*) that such proteins may be a part of the humoral regulatory system that controls the flow of information from nucleic acids. Sequences rich in guanylic acid residues in cellular RNA and DNA have been identified as being involved in such regulations (*5*). Malignant transformation is generally considered to be the result of breakdown of cellular regulatory mechanisms (*6*), and transcription of cellular genome in nerve cells is believed to be an important step during learning (*7*). If the CSF proteins that bind nucleic acids control the informational output from the cellular genome, the observation that the poly(rG)-reacting protein increased in brain malignancies and that the DNA-reacting protein increased in patients with apparent mental disturbances may be of special significance for our understanding of the etiology and the course of these ailments. Whatever the significance of these proteins, our data indicate that the variations in their concentrations are diagnostically significant and that the described assay may be useful for the diagnosis and differential diagnosis of diseases of the central nervous system.

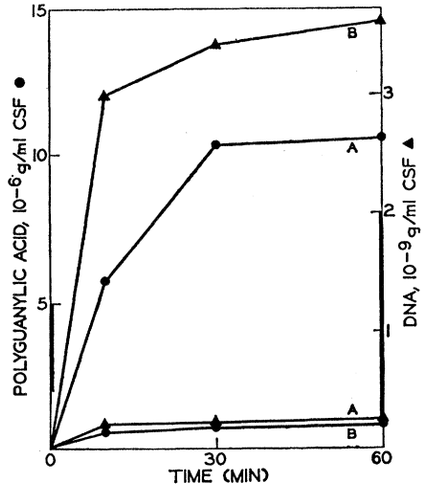

Fig. 1. Binding of *Escherichia coli* [^{32}P]-DNA (triangles) and [^{3}H]poly(rG) (circles) to samples of CSF from a patient with brain tumor (meningioma, case A) and from a patient with hydrocephalus (case B). The heavy lines on both sides of the graph show the approximate range of values for CSF's from apparently normal donors. One microgram of nucleic acid (*8*), labeled with ^{3}H or ^{32}P (10^{4} to 10^{6} count/min) was mixed on ice with 50 μl of CSF sample, NaCl (up to 0.1*M* final concentration) and phosphate buffer (*p*H 7.2, final concentration 0.02*M*). Double-distilled water was added up to 0.5 ml. After mixing the sample was transferred to a 40°C water bath at time zero. At various times (up to 60 minutes) 50-μl samples were withdrawn into 2 ml of 0.1*M* NaCl, 0.1 percent sodium dodecyl sulfate; after 2 minutes of further incubation at room temperature, the samples were transferred onto nitrocellulose membrane filters (Schleicher and Schuell, grade B6) and filtered under moderate vacuum (New Brunswick Scientific Co., model DNA-40 filtration apparatus). The filters were subsequently washed with additional amounts of 0.14*M* NaCl containing 0.05 percent sodium dodecyl sulfate and dried. The radioactivity retained on filters was measured in a low-background gas flow proportional counter or in a scintillation counter.

H. KUBINSKI
MANUCHER JAVID
Division of Neurological Surgery,
University Hospitals,
Madison, Wisconsin 53706

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8. The results obtained with labeled human and bacterial DNA's were quite comparable. Thus, for reasons of economy, bacterial DNA was used for our assays. DNA was obtained from *Escherichia coli* cells labeled (*4*) for more than ten generations with ^{32}P and extracted by a chloroform-butanol-phenol technique (*5*). Other methods commonly used for DNA extraction were less satisfactory. The DNA binds more reproducibly if it is incubated briefly with formaldehyde (1 percent; 10 minutes at 40°C) and then further purified by gel chromatography on Sephadex G25 (Pharmacia, Uppsala). ^{3}H-Labeled poly(rG) was purchased from Miles Laboratories (Elkhart, Ind.). About 90 percent of our assays were done with their lot 32. However, lot 1, which was available later, was successfully used in about 10 percent of our assays. Unfortunately, some lots of poly(rG) from Miles and from other sources have been unsuitable, presumably because the molecular weights were lower than that of lot 32 as measured by velocity sedimentation in sucrose gradients. In collaboration with another supplier (Nuclear Dynamics, El Monte, Calif.) the optimal characteristics for poly(rG) were established, and the product is now available from this manufacturer also.
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