

The Origin of Cerebrospinal Fluid Procoagulant Activity

By Diane M. Komp, Lionel P. Clyne, and Edmund Sullivan

The cerebrospinal fluid of children treated with CNS prophylaxis for acute lymphoblastic leukemia contains a procoagulant that is cell-associated and behaves like tissue

factor. This activity cannot be demonstrated in a platelet-poor system.

GRAEBER AND STUART¹ reported an increase in procoagulant activity in the cerebrospinal fluid (CSF) of children with acute lymphoblastic leukemia (ALL) following prophylactic central nervous system (CNS) treatment with cranial radiation and intrathecal methotrexate. They postulated that the shortening of the plasma recalcification time (PRT) reflected the presence of brain phospholipid released into the CSF by demyelination.

The purpose of the study reported here was to examine in more detail the phenomenon they described. We offer an alternative postulate for the origin of "CSF procoagulant."

MATERIALS AND METHODS

Substrate Plasmas

All blood samples were collected into plastic syringes and processed entirely with plastic until time of use. Blood was anticoagulated with 3.8% sodium citrate in a ratio of nine parts whole blood to one part anticoagulant unless otherwise specified.

Centrifugations at 4300 g or less were performed in an International Centrifuge Model PR-6. Ultracentrifugation was performed in a Sorvall Superspeed RCZ-B machine. In the text, substrate plasmas will be referred to as follows: standard plasma—500 g for 15 min; "platelet-poor" plasma—4300 g for 30 min; ultracentrifuged plasma—39,000 g for 30 min.

Plasmas from individuals with factor VIII or factor IX deficiencies (<1%) were prepared as standard plasma above. Factor V deficient plasma was prepared by the method of Biggs.² Plasma congenitally deficient in factors, II, VII, X, XI, or XII were purchased from George King (Overland Park, Kans.). The plasmas were prepared by centrifugation at 4000 rpm for 20 min when freshly drawn from the donors into 0.1 M buffered citrate anticoagulant.¹

Millipore filtration was performed on 5-ml aliquots of standard plasma using 25-mm Nucleopore filters (Pleasanton, Calif.) with 0.2- μ m pores.

Cerebrospinal Fluids

CSF was collected into plastic tubes. Cyto-centrifugation was performed on fresh samples using a Shandon-Elliot Cytospin, Mark

II (Shandon Southern Scientific, Sewickley, Pa.). Samples of 0.5 ml were centrifuged at 1000 rpm (135 g) for 1.5 min, centrifugation interrupted, and residual fluid aspirated from the sample chamber which tilts away from the vertical at rest. The centrifugation was then completed (additional 3.5 min) so that the side arm was clear of fluid before the slide was removed for staining with Wright-Giemsa as previously described.^{4,6} This centrifuge spins cells through the fluid onto glass slides. With a fluid the viscosity of normal saline (i.e., CSF), most cells of approximately 5 μ diameter will adhere to the slide during the first 1/2 min.⁷ The additional centrifugation time is used to complete the adsorption of fluid onto the filter card.

Recalcification Times

Coagulation tests were performed in triplicate using a Fibrometer (FibroSystem, BioQuest Division, Becton-Dickinson, Cockeysville, Md.). Tests used 0.1 ml substrate plasma, 0.1 ml blank or CSF, and 0.1 ml 0.025 M CaCl₂ unless otherwise indicated. Plasma substrates and CSF were incubated for 1 min prior to performance of recalcification times.

RESULTS

Effect of Centrifugation of Substrate Plasma

Blood of 20 normal donors was pooled after centrifugation at 500, 4300, or 39000 g. Recalcification times were performed after 3-min glass activation. The CSF from five children who were completing cranial radiation and intrathecal therapy were substituted for saline (control) and tested using each of the variously centrifuged plasmas as substrate. Table 1 summarizes the inability of these "active" CSFs to shorten the recalcification time of ultracentrifuged and filtered plasma.

Correction of Congenitally Deficient Plasmas

PRTs were performed after 3-min glass activation upon plasmas congenitally deficient in II, VII, VIII, IX, X, XI, and XII and artificial factor-V-deficient plasma substituting active CSFs for saline. Table 2

Table 1. Effect of Substrate Plasma Centrifugation on CSF Procoagulant Expression

CSF Sample No.	Standard (sec)	Plasma Recalcification Time		
		Platelet Poor (sec)	Ultra centrifuged (sec)	Filtered (sec)
Saline	110.9	201.4	348.4	331.2
353	91.3	180.4	330.1	329.4
354	86.4	179.6	342.9	332.3
355	81.9	162.8	352.1	340.8
356	82.4	180.1	351.9	330.8
357	76.9	163.4	310.9	319.8

From the Departments of Pediatrics and Laboratory Medicine, Yale University School of Medicine, New Haven, Conn.

Supported in part by Yale Comprehensive Cancer Center Grant CA 16359.

Submitted November 8, 1981; accepted August 30, 1982.

Address reprint requests Dr. Diane M. Komp, Department of Pediatrics, Yale University School of Medicine, Room 4087 LMP, 333 Cedar Street, New Haven, Conn. 06510.

© 1983 by Grune & Stratton, Inc.

0006-4971/83/6102-0009\$01.00/0

Table 2. Correction of PRT of Congenitally Deficient Plasma by CSF Procoagulant

Substrate Plasma	Plasma Recalcification Time	
	Saline (sec)	CSF (sec)
Normal	149.4	78.8
II Deficient	222.9	219.5
V Deficient	198.4	201.4
VII Deficient	151.8	150.9
VIII Deficient	314.9	116.3
IX Deficient	276.4	119.2
X Deficient	161.0	158.9
XI Deficient	298.3	209.6
XII Deficient	>400	263.1

presents the average of five "active" CSFs. Shortening was seen of VIII, IX, XI, and XII deficient plasmas but not, however, those deficient in II, V, VII, or X.

Effect of CSF Filtration

Five active CSF samples were Millipore filtered. Separate aliquots of the same samples were cytocentrifuged with a short spin as described in the methods section and the "supernatants" aspirated. Table 3 presents the results of plasma recalcification times using these CSFs. The removal of cells by either filtration or cytocentrifugation diminished the procoagulant activity. Figure 1 demonstrates the typical mononuclear cells found in the active CSF samples.

DISCUSSION

The more effective removal of platelets by either ultracentrifugation or Millipore filtration rendered the

Table 3. Effect of Cellular Removal on Expression of CSF Procoagulant Activity

CSF Sample No.	Saline Control (sec)	Whole CSF (sec)	Plasma Recalcification Time Supernatant	
			Nucleopore (sec)	Cytocentrifuge (sec)
233	130.1	86.4	120.4	131.6
234	130.1	91.2	129.2	129.4
235	130.1	76.2	110.4	126.5
236	130.1	78.5	119.2	132.1
237	130.1	89.1	111.9	130.2

substrate plasma insensitive to the CSF procoagulant. Aoki and von Kaulla⁸ and Caldwell⁹ have defined the necessity for either platelet factor 3 or phospholipids in the system where urinary procoagulant expresses its activity. Although many authors define "platelet-poor plasma" at speeds of less than 5000 *g*,^{8,10,11} Surgenor et al.¹² stated that speeds of 81,000 *g* for 45 min are required to completely remove platelet activity. Biggs² emphasizes the platelet variability as a major problem with the plasma recalcification time as a clinical tool, normal ranges extending from 80 to 250 sec. Graeber and Stuart¹ limited their choice of substrate plasmas to those in the middle of that range (150–180 sec) and prepared their plasma by centrifugation at 1800 *g* for 15 min.¹³

Tissue factor is a lipoprotein complex.¹⁴ It is possible, therefore, that the cell-associated protein component is present in the CSF, which then combines with platelets when they are present in plasma to form a whole tissue factor complex. It has been demonstrated

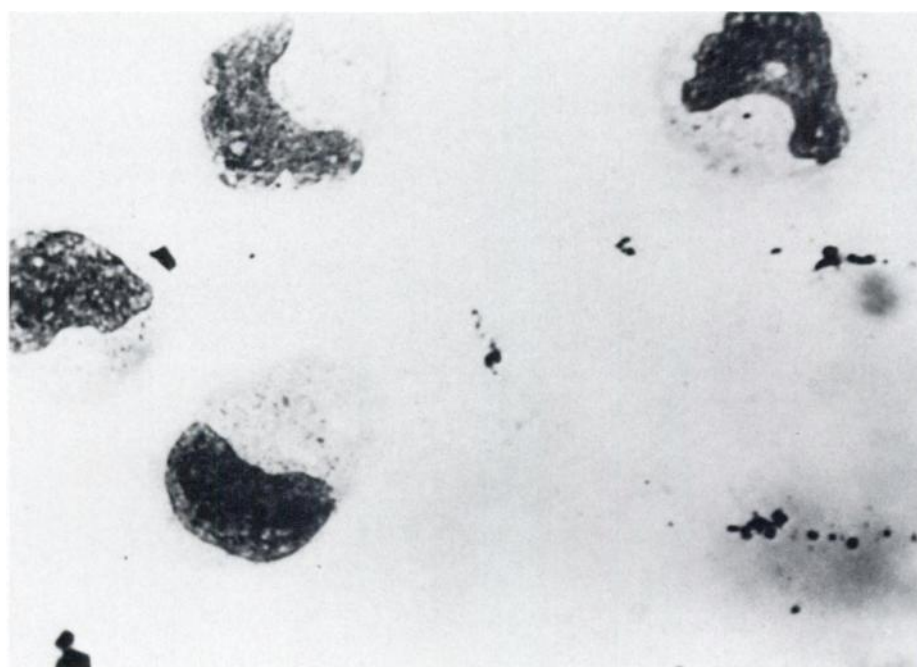


Fig. 1. Mononuclear cells from cytocentrifuged preparation of "active" CSF.

by others^{15,16} that when the lipid portion is removed from tissue factor, it loses its capacity to activate the extrinsic pathway.

We concur with Graeber and Stuart¹ that the CSF of children with ALL who have received preventive central nervous system treatment is capable of shortening the plasma recalcification time but offer an alternative hypothesis to the release of brain phospholipids from demyelination; lumbar punctures, intrathecal therapy, or radiation therapy is responsible for the presence of mononuclear cells in the CSF capable of expressing tissue factor activity.

Rickles et al.¹⁷⁻¹⁹ have described tissue factor generation by monocytes in response to antigens, mitogens, and endotoxin with the assistance of lymphocytes. Monocytoid cells are normally found in a proportion of three monocytoid cells to seven lymphocytes in the CSF.²⁰ Sayk²¹ has referred to reactive conditions with a relatively increased proportion of monocytoid cells as

“subacute irritation syndrome.” Oehmichen²⁰ has observed massive migration of monocytoid cells into the CSF space after repeated lumbar punctures.

Although CSF cell counts did not correlate with CSF procoagulant activity in Graeber’s study, chamber count methodology is insensitive to detection of the small numbers of cells that can be concentrated by cyto centrifugation.^{4,5} Their study and the report by Brueton et al.²² demonstrate similar findings in the CSF of children with infectious meningitis.

A similar cell-associated phenomenon has been described by Zarcharski and Rosenstein²³ for salivary tissue factor that requires factor VII as a cofactor and is diminished by removal of the mucosal epithelial cells by centrifugation. The Millipore and Cytospin removal of cells from our spinal fluid samples accomplished the same effect in our system, supporting the hypothesis that the “CSF procoagulant” is a similar cell-associated tissue factor.

REFERENCES

1. Graeber JE, Stuart MJ: Spinal-fluid procoagulant activity: A sensitive indication of central nervous-system damage. *Lancet* 2:285-288, 1978
2. Biggs R (ed): *Human Blood Coagulation, Haemostasis and Thrombosis* (ed 2). Oxford, Blackwell, 1976
3. George King Co: Personal communication
4. Komp DM: Cyto centrifugation in the management of central nervous system leukemia. *J Pediatr* 81:992-994, 1972
5. Komp DM: Diagnosis of CNS leukemia. *Am J Pediatr Heme Oncol* 1:31-35, 1979
6. Pochedly C: *Leukemia and Lymphoma in the Nervous System*. Springfield, Charles C Thomas, 1977
7. Shandon Scientific, Sales Manual
8. Aoki N, von Kaulla KN: Partial purification and characterization of urinary procoagulant. *Thromb Diath Haemorrh* 26:235, 1971
9. Caldwell J: Procoagulant material from human urine in prothrombin activation. *Thromb Diath Haemorrh* 9:53, 1963
10. Berkowitz RM, Nemerson Y: Quantification of contact system activation using a radiometric assay for activated factor IX. *Blood* 55:528, 1980
11. Niemetz J: Coagulant activity of leukocytes. Tissue factor activity. *J Clin Invest* 51:307, 1972
12. Surgenor DM, Steele BB, Wallach DFH: Thrombin formation in purified systems. *Thromb Diath Haemorrh* 3:214-226, 1959
13. Stuart MJ: Personal communication
14. Nemerson Y, Esnouf MP: Activation of a proteolytic system by a membrane lipoprotein: Mechanism of action of tissue factor. *Proc Natl Acad Sci USA* 70:310-314, 1973
15. Nemerson Y: The phospholipid requirement of tissue factor. *J Clin Invest* 47:72, 1968
16. Deutsch E, Irsigler K, Lomoshitz H: Studien über Gewebethromboplastin 1. Reinigung-chemische Charakterisierung und Trennung in einen Eiweiß- und Lipidanteil. *Thromb Diath Haemorrh* 12:12, 1964
17. Rickless FR, Levin J, Hardin JA, Barr CF, Conrad ME: Tissue factor generation by human mononuclear cells: Effects of endotoxin and dissociation of tissue factor generation from mitogenic response. *J Lab Clin Med* 89:792-803, 1977
18. Edwards RL, Rickles FR, Bobrove AM: Mononuclear cell tissue/factor: Cell of origin and requirements for activation. *Blood* 55:359-370, 1979
19. Rickless FR, Hardin JA, Pitlick FA, Hoyer LW, Conrad ME: Tissue factor activity in lymphocyte cultures from normal individuals and patients with hemophilia A. *J Clin Invest* 52:1427-1433, 1973
20. Oehmichen M: *Cerebrospinal Fluid Cytology*. Philadelphia, W.B. Saunders, 1976
21. Sayk J: *Cytologic der Cerebrospinalflüssigkeit* VEB, Fischer, Jena 1960 as cited by Oehmichen M: *Cerebrospinal Fluid Cytology*. Philadelphia, Saunders, 1976
22. Brueton MJ, Breeze GR, Stuart J: Fibrin-fibrinogen degradation products in cerebrospinal fluid. *J Clin Pathol* 29:341-344, 1976
23. Zacharski LR, Rosenstein R: Reduction of salivary tissue factor (thromboplastin) activity by warfarin therapy. *Blood* 53:366-374, 1979