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Cerebrospinal Fluid and Peripheral White Blood Cell Response to Acute Cerebral Ischemia

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ABSTRACT: We prospectively evaluated the inflammatory response to acute cerebral ischemia in 57 patients who were seen within 72 hours of ictus. All subjects had cerebrospinal fluid examination, complete blood count, sedimentation rate determination, and body temperature monitoring. Correlation analysis was done between these measurements and infarct volume, which was determined by computed tomography of the brain. We found a positive linear correlation between infarct size and the peripheral white blood cell count, specifically the polymorphonuclear leukocyte count. A relationship was also observed for the cerebrospinal fluid protein level, the gamma globulin level, and the cerebrospinal fluid/serum albumin ratio. The correlations observed presumably reflect the extent of tissue injury and secondary inflammatory response in acute cerebral ischemia.

THE ACCUMULATION of neutrophils (polymorphonuclear leukocytes) and monocytes in cerebral infarction is well recognized from autopsy results and from study of middle cerebral artery occlusion in animal models.[1-3] A relationship has been reported between an elevated peripheral white blood cell (WBC) count at 72 hours after stroke onset, larger cerebral infarct size, and adverse clinical outcome.[4] In addition, a transitory rise in the neutrophils in the cerebrospinal fluid (CSF) is seen 3 to 4 days after stroke.[5] There continues to be interest in the role of the inflammatory response in acute stroke[6,7] and the role of neutrophils and monocytes in the pathogenesis of cerebral infarction is of potential clinical relevance.[8,9]

Small variations in body and brain temperature are known to significantly affect tissue damage in animal models of focal and global ischemia.[10] Whereas intraischemic hypothermia provides protection,[11] mild elevation in brain temperature has been shown to worsen histopathologic outcome.[12] Clinically, the importance of small variations in body temperature on ischemic outcome has not been investigated, but it is possible that body temperature in acute stroke reflects the inflammatory response.

The aim of this study was to investigate the relationship between the peripheral and CSF leukocyte response and erythrocyte sedimentation rate (ESR), as well as CSF protein value and body temperature, to the extent of acute cerebral infarction. We included patients with transient ischemic attack (TIA) in this study because cerebral ischemia is a relative phenomenon,[13] and patients with TIA are often found to have evidence of infarction by computed tomography of the brain.[14] A total of 57 patients with acute cerebral ischemia were studied, and all had serial temperature monitoring, admission complete blood count (CBC), ESR determination, and CSF examination.

SUBJECTS AND METHODS

We prospectively assessed the results of the CSF examination, the peripheral WBC count and the body temperature in 338 consecutive patients who came to our hospital within 72 hours of the onset of symptoms of acute stroke or TIA between July 1, 1991, and September 30, 1992. The diagnosis of stroke or TIA was based on standard criteria.[15] High resolution computed tomography (CT) of the brain was done on all patients.

We excluded patients who were found to have primary intracerebral hemorrhage or subarachnoid hemorrhage and patients who

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did not have a CSF examination. The CSF examination was primarily obtained in stroke patients who had positive results of a fluorescent treponemal antibody absorption (FTA-ABS) test to exclude meningovascular syphilis. Despite a previously reported seropositivity rate of 34% in our stroke population,[16] only 0.4% were found to have meningovascular syphilis, and this one patient had antibodies to human immunodeficiency virus (HIV). Thus we felt confident that if the CSF results were not compatible with meningovascular syphilis, then this diagnostic possibility was effectively excluded.

All subjects were considered to have latent syphilis and we eliminated the patients with systemic infection, central nervous system infection, or immunocompromise. We also excluded patients taking nonsteroidal anti-inflammatory agents, as well as those with acute ischemic heart disease, as these factors could also alter the peripheral WBC count. The remaining 57 patients, 38 men and 19 women, had a mean age (± SEM) of 55.2 ± 14.6 years. Six subjects had TIA and 51 had brain infarction.

By CT, the low attenuation area was defined as the infarct and the volume of the hypodense zone was calculated by a planimetric measuring technique.[17,18] When the initial CT was negative for acute infarction, the scan was repeated within 2 to 5 days, and these results were used to calculate infarct size, if an infarct was shown. On the basis of the infarct volume, four groups were delineated: no infarct, small infarct (infarct volume ≤ 10 cm^3), medium infarct (infarct volume ≤ 50 cm^3), and large infarct (infarct volume > 50 cm^3).

The presence of hypertension, diabetes mellitus, cardiac disease, and smoking was determined for each group. On the basis of serial CT findings, we distinguished primary ischemic from primary hemorrhagic stroke, as well as hemorrhagic transformation. We did not exclude subjects with hemorrhagic transformation because this is not an uncommon finding by serial brain scans, and it is usually associated with larger infarcts.[19]

A CBC with differential and ESR were routinely obtained within 6 hours of admission to our medical center. A lumbar puncture (LP) was done within 10 days of admission. The mean interval from time of ictus was 5.7 days. In most instances, the CSF examination was obtained within 4 days of admission. In the 12 subjects with large cerebral infarcts, however, the LP was delayed for 7 to 10 days because of potential mass effect. If the LP was traumatic, the CSF WBC count was corrected under the assumption that the ratio of WBCs to RBCs contaminating the CSF is the same ratio as exists in the peripheral blood and is calculated as follows: CSF WBC corrected = CSF WBC-(serum WBC x CSF RBC/serum RBC).[6]

The CSF protein level was also corrected if the LP was traumatic, with the estimation that 700 red blood cells could explain a 1 mg/dL elevation of the CSF protein. For the 46 patients in whom albumin and gamma globulin (IgG) levels were measured simultaneously in CSF and serum, the CSF/serum albumin ratio and the CSF IgG index were calculated. The CSF/serum albumin ratio (CSF albumin/serum albumin x 10^3) has been reported to reflect blood-brain barrier function.[20] The IgG level in CSF was evaluated by the CSF IgG index equal to (CSF IgG/serum IgG)/(CSF albumin/serum albumin). The CSF IgG index corrects for fluctuations in the serum IgG and reflects the integrity of the blood-brain barrier.[21]

The body temperature was measured on admission and at the time of lumbar puncture. The maximum in-hospital body temperature and the temperature at the time of discharge were also determined.

Using Pearson's correlation analysis,[22] we assessed for a possible relationship between infarct volume and the following: peripheral WBC count, neutrophil count, lymphocyte count, monocyte count, CSF WBC count, CSF protein level, CSF albumin level, CSF IgG level, CSF/serum albumin ratio, CSF IgG index, ESR, and body temperature. By preliminary analysis, a relationship was readily observed for the peripheral WBC count, neutrophil count, CSF protein level, CSF IgG level, and CSF/serum albumin ratio. Only these measurements were used for further analysis. To correct for multiple comparisons on the same data set,[23] we set the α significance level at .01.

RESULTS

Of the 57 patients evaluated, 13 had no lesion on CT, 17 had small infarcts, 15 had moderate infarcts, and 12 had large infarcts. Table 1 shows age, sex, prevalence of stroke risk factors, and frequency of hemorrhagic infarct on CT in each group. There was a higher prevalence of both heart disease and hemorrhagic transformation in the large infarct group.

TABLE 1. Comparison of Age, Sex, Stroke Risk Factors, and CT Findings Among the Four Groups Classified According to Infarct Volume

Group	No.	Age (Mean ± SEM)	Sex (M/F)	Stroke Risk Factors				Hemorrhagic Infarct by CT (%)
				HT (%)	DM (%)	SM (%)	HD (%)	
No lesion	13	48.9 ± 16.3	8/5	7 (53)	1 (8)	6 (69)	0	0
Small infarct	17	51.0 ± 10.7	12/5	12 (71)	3 (18)	12 (71)	3 (18)	1 (6)
Medium infarct	15	58.1 ± 10.5	10/5	11 (73)	4 (27)	11 (73)	4 (27)	2 (13)
Large infarct	12	64.5 ± 16.7	8/4	9 (75)	3 (25)	8 (67)	5 (42)	5 (42)

HT = hypertension; DM = diabetes mellitus; SM = smoking; HD = heart disease.

Table 2 summarizes the correlation analysis of infarct size and its relationship to peripheral WBC count, neutrophil count, lymphocyte count, monocyte count, CSF parameters, ESR, and body temperature. We found a statistically significant positive linear relationship between infarct volume and peripheral WBC count ($r = .405$, $df = 55$, $P < .005$) (Fig 1). Ten subjects (18%) had a total peripheral WBC count >10,000/mm^3, but none of these patients had evidence of infection to explain the leukocytosis. In addition, a significant positive linear correlation was noted for neutrophil count ($r = .515$, $df = 55$, $P < .001$) (Fig 2). On the other hand, there was a trend toward a negative relationship for lymphocyte count ($r = -.302$, $df = 55$, $P < .02$). No significant relationship was observed for the monocyte count. If we exclude the subjects with TIA, the correlations were still present for the peripheral WBC count ($r = .405$, $df = 49$, $P < .005$) and for the neutrophil count ($r = .504$, $df = 49$, $P < .001$).

Six of the 57 subjects had a traumatic lumbar puncture that required correction. Twenty-eight subjects had no WBCs in the CSF, 22 patients had 1 to 4 WBCs (all monocytes), and 7 patients had more than 5 WBCs, the highest cell count being 12 (10 monocytes, 2 leukocytes). No significant correlation was noted between infarct volume and CSF WBC count.

In assessing the values of total protein, albumin, and IgG in the CSF, we excluded the 11 patients with diabetes mellitus because the occurrence of an elevated CSF protein level in patients with diabetes mellitus, especially diabetic neuropathy, is well established.[24,25] Furthermore, we eliminated the 13 patients with no infarct shown by CT in this analysis. Of the remaining 33 patients, the mean value of the CSF protein level was 50.4 ± 23.3 (mean ± SEM, range = 14 to 133). A relationship was noted between infarct volume and both CSF protein ($r = .441$, $df = 31$, $P < .02$) and CSF IgG levels ($r = .495$, $df = 21$, $P < .02$), but this was not statistically significant. A trend was also noted for the CSF/serum albumin ratio ($r = .477$, $df = 21$, $P < .05$), but there was no significant relationship for the CSF IgG index.

DISCUSSION

The role of neutrophils and monocytes in the pathogenesis of ischemic brain injury is controversial and requires further elucidation.

TABLE 2. Summary of Correlation Analysis of Relationship Between Infarct Volume and Peripheral WBC Count, Cerebrospinal Fluid Parameters, Body Temperature, and ESR

	Mean ± SEM	Range	r	df	P Value†
Peripheral WBC count (/mm^3)	8,280 ± 3,030	3,000 - 20,200	0.405	55	< .005
Neutrophils (/mm^3)	5,160 ± 1,870	1,140 - 16,000	0.515	55	< .001
Lymphocytes (/mm^3)	2,420 ± 780	760 - 5,370	- 0.302	55	< .02
Monocytes (/mm^3)	470 ± 250	0 - 1,645	0.002	55	NS
CSF WBC count (/mm^3)	1.7 ± 2.4	0 - 12	0.152	55	NS
CSF protein level (mg/dL)	50.4 ± 23.3	14 - 133	0.441	31	< .02
CSF albumin level (mg/dL)	24.3 ± 9.0	10.1 - 43.1	0.401	21	NS
CSF IgG level (mg/dL)	5.5 ± 3.4	2.5 - 15.1	0.495	21	< .02
CSF/serum albumin ratio	6.80 ± 3.28	3.42 - 17.84	0.477	21	< .05
CSF IgG index	0.53 ± 0.12	0.34 - 0.78	0.303	21	NS
Body temperature on admission (°F)	98.4 ± 1.1	94.2 - 100.2	0.031	55	NS
At LP (°F)	97.9 ± 0.8	96.2 - 100.0	0.053	55	NS
At maximum (°F)	99.5 ± 0.8	98.0 - 102.4	0.244	55	NS
At discharge (°F)	97.9 ± 1.0	95.5 - 101.4	0.259	55	NS
ESR (mm/hr)	14.5 ± 6.8	2 - 36	0.153	49	NS

r = Sample correlation coefficient; df = degree of freedom; NS = not significant; LP = lumbar puncture; ESR = erythrocyte sedimentation rate.

†Significance level is at $P < .01$ to correct for mutiple comparisons.

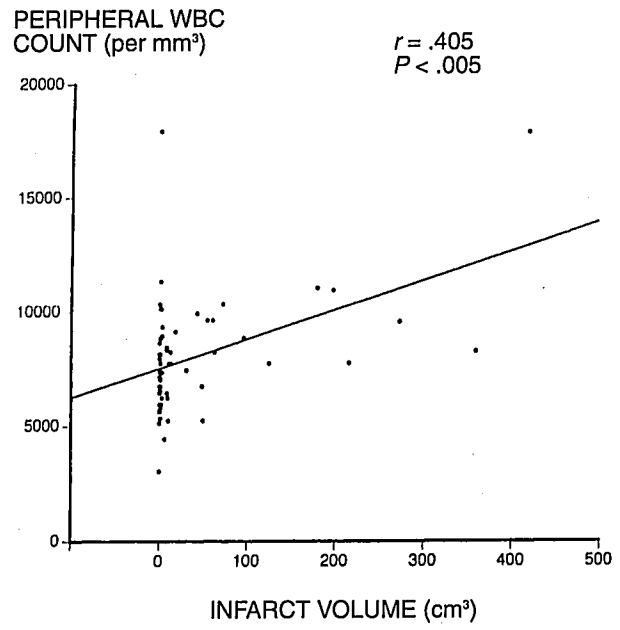

FIGURE 1. Relationship between infarct volume (in cubic centimeters) and peripheral white blood cell (WBC) count.

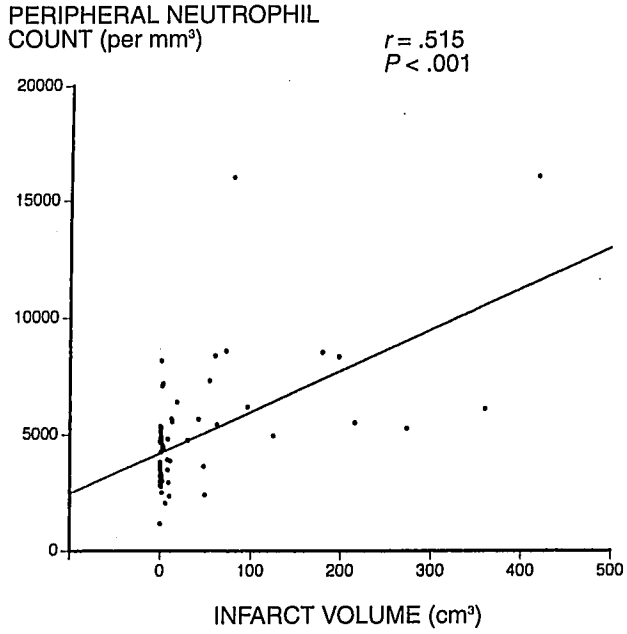

FIGURE 2. Relationship between infarct volume and peripheral neutrophil count.

It has been suggested that leukocytes play a pivotal role in the ischemic process and that therapy designed to suppress this white blood cell response may be of potential value.[9]

Our study shows a positive linear relationship between infarct volume, as measured by CT of the brain, and peripheral WBC count, specifically the neutrophil count. The negative linear relationship for the peripheral lymphocyte count would appear to reflect the shift, or increase, in neutrophil count at the expense of the lymphocytes. Since this study excluded subjects whose medical condition could potentially alter the immune response, the elevation of the neutrophil count in our patients would appear to reflect the primary inflammatory response to acute cerebral ischemia. It is also possible that a stress-induced response on the circulating level of endogenous corticosteroid is involved in the neutrophil reaction that was observed.[26] Increased neutrophil counts are usually regarded as a nonspecific indicator of infection, inflammation, or physical or emotional stress.[27] The potential for increased endogenous corticosteroid promoting a significant neutrophil response in acute ischemic stroke has not been evaluated, to our knowledge, and remains subject to speculation.

By histopathologic study in the squirrel monkey,[1] neutrophil infiltration is observed as a "reactive zone" in the inflammatory response to acute infarction induced by clipping of the middle cerebral artery. A similar type of inflammatory response, with infiltration of neutrophils, has been observed in humans.[2,3,5] In vivo use of radionuclide-labeled WBCs has allowed imaging of this inflammatory process by nuclear scan.[28,29] Recent experimental studies of focal or global ischemia have revealed the accumulation of neutrophils in the microvessels and parenchyma within 24 hours of vascular occlusion.[12,30,31] Garcia and Kamijyo[1] found the maximal neutrophil accumulation at 48 to 72 hours after ictus. It is of concern that these neutrophils release lysosomal enzymes that can transform molecular oxygen into superoxide anions, hydrogen peroxide, and hydroxyl radical (ie, free radicals) in the peripheral tissue surrounding the infarct.[32,33]

Fishman et al[34] reported that the crude membrane fraction of leukocytes can induce cytotoxic edema with increased anaerobic glucose utilization and secondary lactate production accompanied by energy depletion. Such a process could augment tissue injury in the surrounding penumbra. In a recent experimental study of atherosclerosis in primates, activated neutrophils were observed to increase resistance in large cerebral arteries.[35] In addition, the accumulation of leukocytes in a lesion induced by a low-flow state has been detected in the first hours after ischemia, and this suggests an early contribution from these cells in the hemostatic and inflammatory process.[30] In stroke patients, leukocyte adhesion promotes

the endothelial components laminin and fibronectin, which suggests that circulating neutrophils in ischemic stroke may contribute to impairment of microvascular flow.[36]

Pozzilli et al[4] found that an elevated peripheral WBC count, seen at 72 hours after the onset of stroke, was associated with poor clinical outcome. These authors did not evaluate the relationship to the differential WBC count, however. Our study suggests that it is specifically the neutrophils that reflect the inflammatory response that correlates with infarct volume.

Our results also show that the CSF protein level tends to correlate with the extent of brain damage. The lack of a clear-cut relationship between infarct volume and specific CSF protein components presumably represents leakage of the protein from a disrupted blood-brain barrier or age-related changes in CSF protein level.[37] The elevated CSF IgG level may reflect an immune-mediated component, however. It is not unexpected that the larger the infarct, the more severe the disruption of the blood-brain barrier.[25] Although we did not specifically address infarct mechanism, large infarcts were more frequently associated with heart disease and hemorrhagic transformation. Sornas et al[5] have reported that hemorrhagic transformation is associated with a more pronounced neutrophil reaction. It was recently proved that neutrophils migrate across the blood-brain barrier via a transendothelial route, with augmented disruption of the blood-brain barrier by the acute inflammatory response induced by α-bungarotoxin.[38] The protein leakage into the CSF may be facilitated by activated neutrophils during evolution of the ischemia.

It is possible that the activated neutrophil response, after acute cerebral ischemia, may contribute to ongoing tissue injury, especially in the surrounding penumbra. In this regard, an elevated leukocyte count has been reported to increase the risk of cerebral infarct and TIA.[39,40] Thus the inflammatory response to acute cerebral ischemia might not just be an epiphenomenon, but could play a critical role in the pathogenesis.

References

1. Garcia JH, Kamijyo Y: Cerebral infarction: evolution of histopathological changes after occlusion of a middle cerebral artery in primates. *J Neuropathol Exp Neurol* 1974; 33:408-421
2. Adams RP, Sidman RL: *Introduction to Neuropathology.* New York, McGraw-Hill Book Co, 1968, pp 172-175
3. Graham DI: Hypoxia and vascular disorders. *Greenfield's Neuropathology.* Adams JH, Duchen LW (eds). New York, Oxford University Press, 9th Ed, 1992, pp 195-200
4. Pozzilli C, Lenzi GL, Argentino C, et al: Peripheral white blood cell count in cerebral ischemic infarction. *Acta Neurol Scand* 1985; 71:396-400
5. Sornas R, Ostlund H, Muller R: Cerebrospinal fluid cytology after stroke. *Arch Neurol* 1972; 26:489-501
6. Nadis SM, Klawans H: Vascular diseases. Part II: Cerebrospinal fluid in stroke. *Handbook of Clinical Neurology.* Toole JF (ed). New York, Elsevier Science Publishers Co, 1989, pp 195-202
7. Ramirez-Lassepas M, Patrick BK: Cerebrospinal fluid cellular response in uncomplicated acute ischemic stroke. *J Stroke Cerebrovasc Dis* 1992; 2:168-172
8. Ernst E, Hammerschmidt DE, Bagge U, et al: Leukocytes and the risk of ischemic diseases. *JAMA* 1987; 257:2318-2324
9. Kochanek PM, Hallenbeck JM: Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. *Stroke* 1992; 23:1367-1379
10. Morikawa E, Ginsberg MD, Dietrich WD, et al: The significance of brain temperature in focal cerebral ischemia: histopathological consequences of middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab* 1992; 12:380-389
11. Busto R, Dietrich WD, Globus MY-T, et al: Small differences in intra-ischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab* 1987; 7:729-738
12. Dietrich WD, Busto R, Valdes I, et al: Effects of normothermic versus mild hyperthermic forebrain ischemia in rats. *Stroke* 1990; 21:1318-1325
13. Koudstaal PJ, van Gijn J, Frenken GWGM, et al: TIA, RIND, minor stroke: a continuum or different subgroups? *J Neurol Neurosurg Psychiatry* 1992; 55:95-97
14. Koudstaal PJ, van Gijn J, Lodder J, et al: Transient ischemic attacks with and without a relevant infarct on computed tomographic scans cannot be distinguished clinically. *Arch Neurol* 1991; 48:916-920
15. Classification of cerebrovascular disease. III. Special report from the National Institute of Neurological Disorders and Stroke. *Stroke* 1990; 21:637-676
16. Kelley RE, Bell L, Kelley SE, et al: Syphilis detection in cerebrovascular disease. *Stroke* 1989; 20:230-234
17. Takagi S, Shinohara Y: Internal carotid occlusion: volume of cerebral infarction, clinical findings, and prognosis. *Stroke* 1981; 12:835-839
18. Liliequest B, Wirell S: Quantitative estimation of tumor volume on computer assisted tomography. *J Comput Assist Tomogr* 1978; 2:300-302
19. Hart RG, Easton JD: Hemorrhagic infarcts. *Stroke* 1986; 17:586-589
20. Tibbling G, Link G, Ohman S: Principles of albumin and IgG analyses in neurological disorders: I. Establishment of reference values. *Scand J Clin Lab Invest* 1977; 37:385-390
21. Rostrom B, Link H: Oligoclonal immunoglobulin in cerebrospinal fluid in acute cerebrovascular disease. *Neurology* 1981; 31:590-596
22. Altman DG: Relation between two continuous variables. *Practical Statistics for Medical Research.* London, Chapman and Hall Co, 1991, pp 277-324
23. Kleinbaum DG, Kupper LL, Muller KE: *Applied Regression Analysis and Other Multivariate Methods.* Boston, PWS-Kent Publishing Co, 1988, p 32
24. Ive ER: Protein content in the cerebrospinal fluid of diabetic patients. *Bull Los Angeles Neurol Soc* 1987; 22:95-111
25. Fishman RA: *Cerebrospinal Fluid in Diseases of the Nervous System.* Philadelphia, WB Saunders Co, 2nd Ed, 1992, pp 314-334
26. Bagby GC: Leukocytosis and leukemoid reactions. *Cecil's Textbook of Medicine.* Wyngaarden JB, Smith LH, Bennett JC (eds). Philadelphia, WB Saunders Co, 19th Ed, 1992, pp 914-920

27. David CD: Neutrophilia. *Hematology*. Williams JW, Ernest B, Allan JE, et al (eds). New York, McGraw-Hill Book Co, 4th Ed, 1990, pp 816-820
28. Pozzilli C, Lenzi GL, Argentino C, et al: Imaging of leukocytic infiltration in human cerebral infarcts. *Stroke* 1985; 16:251-255
29. Wong PY, Kao GH, Moi MY, et al: Leukocyte infiltration in acute hemispheric ischemic stroke. *Stroke* 1993; 24:236-240
30. Hallenbeck JM, Dutka AJ, Tanishima T, et al: Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. *Stroke* 1986; 17:246-253
31. del Zoppo GJ, Schmid-Schonbein GW, Mori E, et al: Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke* 1991; 22:1276-1283
32. Weissman G, Smolen J, Korchak HM: Release of inflammatory mediators from stimulated neutrophils. *N Engl J Med* 1980; 303:27-31
33. Fridovich I: The biology of oxygen radicals: the superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense. *Science* 1978; 201:875-880
34. Fishman RA, Sligar K, Hake RB: Effect of leukocytes on brain metabolism in granulocytic brain edema. *Ann Neurol* 1977; 2:89-94
35. Lopez JA, Armstrong ML, Harrison DG, et al: Vascular responses to leukocyte products in atherosclerotic primates. *Circ Res* 1989; 65:1078-1086
36. Grau AJ, Berger E, Paul Sung K-L, et al: Granulocyte adhesion, deformability, and superoxide formation in acute stroke. *Stroke* 1992; 23:33-39
37. Garton MJ, Keir G, Lakshmi M, et al: Age-related changes in cerebrospinal fluid protein concentration. *J Neurol Sci* 1991; 104:74-80
38. Faustman PM, Dermietzel R: Extravasation of polymorphonuclear leukocytes from the cerebral microvasculature: inflammatory response induced by alpha-bungarotoxin. *Cell Tissue Res* 1985; 242:399-407
39. Prentice RL, Szatrowski TP, Kato H, et al: Leukocyte count and cerebrovascular disease. *J Chronic Dis* 1982; 35:703-714
40. Harrison MJG, Marshall J: Does the peripheral blood leukocyte count predict the risk of transient ischemic attacks and strokes? *J Neurol Neurosurg Psychiatry* 1987; 50:1558-1559