Increased Release of Tumor Necrosis Factor-α Into the Cerebrospinal Fluid and Peripheral Circulation of Aged Rats

Anna-Leena Sirén, MD, PhD; Yong Liu, MD; Giora Feuerstein, MD; and John M. Hallenbeck, MD

Background and Purpose: We earlier reported that risk factors for stroke prepare brain stem tissue for a modified Shwartzman reaction, including the development of ischemia and hemorrhage and the production of tumor necrosis factor-α, after a provocative dose of lipopolysaccharide. In the present study, we sought to determine whether blood and central nervous system cells of rats with the stroke risk factor of advanced age produce more proinflammatory and prothrombotic mediators than do those of young rats of the same strain.

Methods: Levels of tumor necrosis factor-α and platelet activating factor in the cerebrospinal fluid and tumor necrosis factor-α in the serum of 2-year-old and 16-week-old Sprague-Dawley rats were monitored before and after challenge with lipopolysaccharide.

Results: No consistent tumor necrosis factor-α activity was found in the cerebrospinal fluid or blood of control animals. Intravenous administration of lipopolysaccharide (1.8 mg/kg) increased serum tumor necrosis factor-α levels but had no effect on tumor necrosis factor-α in the cerebrospinal fluid. Serum tumor necrosis factor-α increased much more in aged rats than in young rats. When lipopolysaccharide was injected intracerebroventricularly, tumor necrosis factor-α activity in cerebrospinal fluid increased significantly more in old rats than in young rats. Baseline levels of platelet activating factor in cerebrospinal fluid were significantly higher in old rats than in young rats, and the levels increased to a greater degree in aged rats on stimulation.

Conclusions: Rats with the stroke risk factor of advanced age respond to lipopolysaccharide with a more exuberant production of tumor necrosis factor-α and platelet activating factor than young rats of the same strain. These findings are consistent with our working hypothesis that perivascular cells are capable of exaggerated signaling of endothelium through cytokines such as tumor necrosis factor-α in animals with stroke risk factors. The effect of such signaling might be to prepare the endothelium of the local vascular segment for thrombosis or hemorrhage in accord with the local Shwartzman reaction paradigm. (Stroke 1993;24:880-888)

Key Words • lipopolysaccharides • platelet activating factor • tumor necrosis factor • rats

Risk factors for stroke, such as aging, hypertension, diabetes, and atherosclerosis, are associated with increased subendothelial accumulation of monocytes and macrophages. Expression of the macrophage-derived cytokines tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 has been demonstrated in human atheromas.

The opinions or assertions expressed are the private ones of the authors and are not to be construed as official or as necessarily reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

From the Department of Neurology (A.-L.S., Y.L., J.M.H.), Uniformed Services University of the Health Sciences, Bethesda, Md.; the Department of Pharmacology (G.F.), SmithKline Beecham Laboratories, King of Prussia, Pa.; and the Stroke Branch (J.M.H.), National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md.

Supported by Public Health Service grant NS-28225.

Address for correspondence: Anna-Leena Sirén, MD, PhD, Department of Neurology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814.

Received May 21, 1992; final revision received October 22, 1992; accepted February 15, 1993.

See Editorial Comment, page 887

We have been interested in the possibility that an interaction of perivascular macrophages with endothelium could contribute to the increased risk of stroke in hypertension and in advanced age. This interaction could be mediated by increased activity of cytokines such as TNF-α and interleukin-1 (IL-1), which are able to increase endothelial adhesion of monocytes, increase procoagulant properties, and suppress anticoagulant properties of the endothelium and circulating blood elements. The altered state of blood vessels induced by stroke risk factors could render such vessels locally vulnerable to thrombosis or hemorrhage in response to transient activation of intravascular coagulation or of complement. The site of thrombus formation would be determined by the preexisting vessel disturbance and not by the intravascular process activating the complement or coagulation systems, thereby following the local Shwartzman reaction paradigm. In an earlier study, we found that several risk factors for stroke, including advanced age, diabetes, hypertension, and genetic stroke-proneness, prepared brain stem tissue for a
modified Shwartzman reaction that involved the development of ischemia and hemorrhage after a provocative dose of lipopolysaccharide administered intracerebrally or intravenously. Furthermore, when challenged with a provocative intravenous dose of lipopolysaccharide, rats with hypertension produced more TNF-α in their blood than did normotensive rats. Hypertensive rats also synthesized and released more TNF-α from brain tissue after the injection of lipopolysaccharide into the lateral cerebral ventricle than did normotensive rats. To determine whether the increased release of inflammatory mediators is associated with stroke risk factors other than high blood pressure, we measured the production of TNF-α and platelet activating factor (PAF), two proinflammatory and procoagulant mediators, in the blood and cerebrospinal fluid (CSF) of aged compared with young normotensive rats. TNF-α was chosen to represent the cytokine class of mediators because it activates endothelium for leukocyte adherence and procoagulant processes. PAF was selected to represent the lipid inflammatory mediators, which induce leukocyte chemotaxis and adhesion and activate prothrombotic processes.

These variables were measured in the serum and CSF under basal and stimulated conditions, with lipopolysaccharide administered intravenously or injected into the lateral cerebral ventricle. Lipopolysaccharide was chosen as the stimulus for the release of TNF-α and PAF because it is a potent activator of cells capable of producing cytokines or PAF.

**Materials and Methods**

Two-year-old (23–24-month-old) and 16-week-old male Sprague-Dawley (Zelienople, Pa.) and housed at 22°C with a 12-hour/12-hour light/dark cycle, with food and tap water ad libitum. The mean±SEM body weight was 85.8±2.1 g for the 2-year-old rats (n=36) and 550±16 g for the 16-week-old rats (n=36). The difference in body weight between the groups was significant (p<0.001, Student’s two-tailed t test).

These experiments were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council (US Department of Health, Education, and Welfare publication No. [NIH] 85-23, 1985).

**Surgical Procedures**

The rats were anesthetized with halothane (4% in 100% oxygen). For intravenous injections, a sterile PE-50 catheter was inserted into the femoral vein under aseptic conditions. Lipopolysaccharide from *Escherichia coli* serotype 0111:B4 (phenol extracted; Sigma Chemical Co., St. Louis, Mo.) dissolved in sterile, pyrogen-free 0.9% NaCl was injected through the catheter. Lipopolysaccharide was administered at three dose levels, 1 µg/kg, 1.8 mg/kg, and 3.6 mg/kg, to reflect its effect at a threshold dose and at a dose that maximally activates TNF-α in rats. After the injection, the catheter was pulled out and the vein ligated. For intracerebroventricular injections, the rat was placed in a stereotaxic device (David Kopf Instruments, Tujunga, Calif.). A midline cut was made on the scalp to expose the parieto-occipital bone area, and the muscles attached to the occiput were gently separated to expose the atlanto-occipital membrane. A stainless-steel guide cannula was placed on the parietal skull (coordinates from bregma: anteroposterior, 0.8 mm; lateral, 1.2 mm) and fixed with glue. Lipopolysaccharide dissolved in sterile, pyrogen-free 0.9% NaCl was injected in a total volume of 10 µL by means of a premeasured 30-gauge cannula (7.5 mm) over a period of 5 minutes. An additional 2 minutes were allowed for dispersal of the injected fluid before withdrawal of the cannula. Because differences in brain volume do not correlate with differences in body weight in adult rats, the intracerebroventricular doses of lipopolysaccharide were calculated per rat rather than per body weight. The three doses used (750 ng per rat, 1.35 mg per rat, and 3.0 mg per rat) corresponded to the intravenous doses of lipopolysaccharide calculated for a body weight of 700 g. Control rats received 10 µL of sterile, pyrogen-free 0.9% NaCl through an intracerebroventricular needle.

Two hours after the injection of saline or lipopolysaccharide, the rats were anesthetized with halothane and placed in a stereotaxic device. A sterile 30-gauge needle attached to a sterile syringe was inserted into the intracisternal space through the atlanto-occipital membrane. Clear CSF in a volume of 150 µL was withdrawn and frozen immediately in a sterile Eppendorf vial on dry ice. Thereafter, a laparotomy was performed, and a sterile 23-gauge butterfly needle was inserted into the abdominal aorta and blood was withdrawn into a sterile syringe. A 2-mL volume of the collected blood was allowed to clot, and serum was collected.

**Tumor Necrosis Factor Assay**

The serum or CSF samples were stored at 20°C for 1–2 weeks until assayed for TNF-α using the L-929 cell lysis bioassay, as described elsewhere. In brief, L-929 cells were grown in supplemented Eagle’s minimum essential medium (MEM)+10% fetal bovine serum (FBS) in 150-mL tissue culture flasks; 8×105 cells were seeded into 50 mL of medium. L-929 cells were plated at 4×105 cells/mL in a 100-µL well and incubated overnight. Before the experiment, the medium was aspirated, and 50 µL of MEM+10% FBS was added to the well. Test samples (25 µL of serum or CSF) underwent twofold serial dilutions by sequential transfer of 50 µL across the wells. In addition, 50 µL of actinomycin D was added to each well to a final concentration of 0.4 µg per well. Incubation was terminated by aspiration of the supernatants and washing with normal saline (200 µL per well).

After aspiration of the saline, the cells were stained by exposure to 50 µL of 0.05% crystal violet in 20% ethanol for 10 minutes. Scoring of the wells was done visually, and TNF-α units were calculated as the reciprocal of the highest dilution giving 50% lysis of a well. To convert to units per milliliter, this value was multiplied by 20.

**Platelet Activating Factor Assay**

A 100-µL aliquot of CSF was used for the PAF radioimmunoassay using a commercial PAF-radioimmunoassay kit (NEK-062, Dupont/NEN Products, Boston, Mass.). The sensitivity of the assay was 50 pg.
White Blood Cell Counts

In a parallel study, rats (10 in each group) were anesthetized with halothane (4% in 100% oxygen). When surgical anesthesia was reached (usually within 3–5 minutes), a laparotomy was performed and a sterile 23-gauge butterfly needle was inserted into the abdominal aorta. A blood sample (1 mL) was then withdrawn into a heparinized sterile syringe. The blood samples were collected in the morning before 10 AM. The total leukocyte count of the freshly withdrawn blood was measured with an automatic cell counter (System 9000, Baker Instruments). The differential leukocyte counts for neutrophils, monocytes, and lymphocytes were made from a blood smear stained with Giemsa-Wright’s stain.

Measurement of Arterial Pressure and Heart Rate

In parallel experiments, rats (four in each group) were anesthetized with halothane, and a sterile PE-50 catheter was implanted into the left femoral artery for continuous monitoring of blood pressure and sampling of arterial blood. The catheters were tunneled under the back skin, exited at the nape of the neck, and secured by a soft spring wire. After the rats had recovered from anesthesia, the arterial catheter was connected to a pressure transducer (Narco Bio-Systems RP 1500i, Houston, Tx.), and blood pressure and heart rate were continuously recorded on the Narcotrace 80 computerized physiograph (Narco) and sampled automatically every 30–60 seconds by a Northstar-Hazeltine computer for 30 minutes. Thereafter, a blood sample (1 mL) was withdrawn from the femoral artery for analysis of blood glucose. A single dose of lipopolysaccharide (1.8 mg/kg) was injected into the femoral arterial catheter, and the catheter was then reconnected to the pressure transducer and blood pressure monitored for an additional 3 hours. A second blood sample (1 mL) for blood glucose measurement was withdrawn 2 hours after lipopolysaccharide administration. The rats were then killed by injecting an overdose of pentobarbital into the femoral arterial catheter. Blood glucose was analyzed using routine methods.

Statistical Analysis

The dose–response relations were analyzed by two-way analysis of variance (ANOVA) using the CSS/PC statistical package for microcomputers (Complete Statistical System, StatSoft Inc., Tulsa, Okla.). When ANOVA revealed significances in the test parameters, Tukey’s test was used to analyze the differences between individual treatment groups.

Results

TNF-α activity in the CSF or serum of control animals was low or absent. The 1.8- and 3.6-mg/kg i.v. doses of lipopolysaccharide increased serum TNF-α levels in a dose-dependent manner, but the low dose of 1 μg/kg had no effect; the increase in serum TNF-α activity was greater in old rats than in young rats (Figure 1, top panel). Time-course studies at the 1.8-mg/kg dose confirmed the previous findings11,14 that the peak response in serum TNF-α activity is reached 2 hours after lipopolysaccharide administration. Thus, the serum TNF-α level in young rats 2 hours after lipopolysaccharide administration was 40.8±23.1×10^3 units/mL (mean±SEM) and declined to 12.8±9.4×10^3 units/mL at 3 hours after lipopolysaccharide administration. In the old rats the corresponding values were 114.3±33.6×10^3 units/mL and 33.3±16.4×10^3 units/mL, respectively. The 3.6-mg/kg i.v. dose of lipopolysaccharide increased the CSF levels of TNF-α in the aged rats, whereas the lower doses had no effect (Figure 1, bottom panel).

With the intracerebroventricular injection of lipopolysaccharide, TNF-α activity was increased in serum and CSF after the 1.35- and 3.0-mg per rat doses, whereas the low dose of 750 ng per rat had only minimal or no effect; at the highest dose the increase in serum TNF-α activity was significantly greater in old rats than in young rats (Figure 2, top panel). After intracerebroventricular administration, the 1.35- and 3.0-mg per rat doses of lipopolysaccharide increased TNF-α activity in the CSF in a dose-dependent manner in the aged rats (Figure 2, bottom panel). The peak response in TNF-α activity in the CSF in the young rats was achieved at the 1.35-mg per rat dose. At both dose levels the increase in CSF TNF-α activity was greater in the aged than the young rats (Figure 2, bottom panel).

The effect of lipopolysaccharide on the level of PAF in the CSF is summarized in Table 1. The basal CSF
broventricular injection of lipopolysaccharide, the CSF level of PAF increased significantly after the dose of 1.35 mg per rat in the old rats, whereas in the young rats the CSF PAF levels did not change significantly after either dose.

In parallel studies, the white blood cell counts in untreated old and young rats ranged from \(3 \times 10^3\) to \(10 \times 10^3\) cells/\(\mu L\). The mean white cell count in the old rats was \(5.7 \pm 0.8 \times 10^3\) and in the young rats \(4.7 \pm 0.8 \times 10^3\) cells/\(\mu L\). The percentages of neutrophils, lymphocytes, and monocytes in blood of old rats \((n=10)\) were \(27 \pm 4\%\) (mean\(\pm\)SEM), \(64 \pm 4\%\), and \(6 \pm 2\%\), respectively. The corresponding cell counts in the young rats \((n=9)\) were \(11 \pm 4\%\), \(84 \pm 4\%\), and \(3 \pm 1\%\), respectively. The total white blood cell counts or the monocyte counts were not significantly different in old rats compared with young rats. However, the old rats' blood contained significantly fewer lymphocytes \((p<0.01,\) Student's two-tailed test for independent samples) and significantly more neutrophils \((p<0.05,\) Student's two-tailed \(t\) test) than the blood of young rats.

The mean arterial pressure, heart rate, and serum glucose levels were measured in six rats. No significant differences were found in the hemodynamic variables or the serum glucose levels between the young and old rats. The mean arterial pressure and heart rate before administration of lipopolysaccharide were \(104 \pm 4\) mm Hg and \(302 \pm 28\) beats per minute, respectively, in the 16-week-old rats. The corresponding values in the 2-year-old rats were \(106 \pm 8\) mm Hg and \(323 \pm 29\) beats per minute, respectively. At 2 hours after an intravenous bolus of 1.8 mg/kg lipopolysaccharide, the blood pressure and heart rate were \(108 \pm 2\) mm Hg and \(319 \pm 15\) beats per minute in young rats and \(103 \pm 2\) mm Hg and \(367 \pm 24\) beats per minute in old rats. The nonfasting serum glucose in young rats was \(299 \pm 20\) mg/dL (mean\(\pm\)SEM, \(n=3\)) and in old rats was \(313 \pm 35\) mg/dL \((n=3)\).

**Discussion**

Sprague-Dawley rats with the stroke risk factor of advanced age produced more TNF-\(\alpha\) in blood and CSF in response to a provocative dose of lipopolysaccharide.

**Figure 2.** Bar graphs show dose–response effect of intracerebroventricular (ICV) lipopolysaccharide (LPS) on serum (top panel) and cerebrospinal fluid (CSF) (bottom panel) tumor necrosis factor (TNF)-\(\alpha\) in 2-year-old and 16-week-old rats. Values (mean\(\pm\)SEM) represent levels of TNF-\(\alpha\) 2 hours after injection of saline or LPS, \(n=5-8\) in each group. *\(p<0.05\) (Tukey's test) compared with 16-week-old rats; +\(p<0.05\) (Tukey's test) compared with saline-treated group.

**Table 1.** Effect of Lipopolysaccharide on Cerebrospinal Fluid Platelet Activating Factor Level in 2-Year-Old and 16-Week-Old Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2-Year-old</th>
<th>16-Week-old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline i.v.</td>
<td>(9,598\pm1,974^*)</td>
<td>9</td>
</tr>
<tr>
<td>LPS (1 (\mu g/)kg)</td>
<td>(9,840\pm1,563^*)</td>
<td>5</td>
</tr>
<tr>
<td>LPS (1.8 mg/kg)</td>
<td>(9,047\pm1,688^*)</td>
<td>6</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>(7,117\pm1,164)</td>
<td>6</td>
</tr>
<tr>
<td>LPS (750 ng per rat)</td>
<td>(6,096\pm716)</td>
<td>5</td>
</tr>
<tr>
<td>LPS (1.35 mg per rat)</td>
<td>(22,920\pm7,359^{+\dagger})</td>
<td>5</td>
</tr>
</tbody>
</table>

Values (mean\(\pm\)SEM) represent platelet activating factor (PAF) levels 2 hours after saline or lipopolysaccharide (LPS) administration. \(n\)=number of rats. CSF, cerebrospinal fluid.

*\(p<0.05\) (Tukey's test) compared with 16-week-old rats.

\(+p<0.05\) (Tukey's test) compared with saline-treated group.

Statistical analysis by two-way analysis of variance indicated significant differences between treatment and age after intracerebroventricular LPS: age \((F=7.93350, p=0.00913)\), interaction \(F=5.54648, p=0.01014\), interaction age\(\times\)treatment \((F=4.45993, p=0.02099)\). Corresponding \(F\) and \(p\) values after intravenous LPS: age \((F=20.36555, p=0.00022)\), treatment \((F=0.49891, p=0.61734)\), interaction age\(\times\)treatment \((F=0.20631, p=0.81489)\).
than did young rats. This species and strain and these ages were chosen to correspond to the susceptibilities to stroke as previously described. The definition of an "old" rodent differs among studies. Most of the studies examining the effect of aging on immune function have used 24-month-old animals as old test subjects. Some studies using 30-36-month-old mice as old subjects contend that the age of 24 months actually represents "middle age" in the rodent life span. The effects of aging on cytokine-producing cells are complex. In agreement with our present data, serum TNF-α activity was increased more than 20-fold in 30-month-old mice compared with 6-month-old mice when the mice were treated with an intravenous injection of streptococcal endotoxin. The isolated murine peritoneal macrophages from 24-month-old mice produced more TNF-α when stimulated with lipopolysaccharide than did the macrophages from 8-month-old mice. Macrophages from Lewis rats older than 24 months had a slightly enhanced production of IL-1 compared with macrophages from 3-4-month-old rats. However, peritoneal macrophages from 36-month-old mice were not significantly different in their TNF-α production than 8-month-old mice of the same strain. Isolated peritoneal macrophages of 12-13-month-old (middle-aged) and 22-23-month-old (old) Fisher rats released less TNF-α than peritoneal macrophages of 2-3-month-old rats when challenged with Staphylococcus epidermidis. Peritoneal macrophages of 24-month-old mice also produced less IL-1 than macrophages from 2-4-month-old mice. Earlier studies have suggested that any impairment in the capacity of macrophages to produce cytokines in vitro might be compensated for in vivo by the increased number of monocytes and macrophages in old animals. In the present study, the monocyte counts in old rats (6 ± 2%) were not significantly different than in young rats (3 ± 1%). Therefore, the increased TNF-α levels in the blood and CSF of old animals stimulated with lipopolysaccharide may indicate an increased capacity of cells from aged rats to produce TNF-α. The studies by Shimada demonstrating an increased in vivo production of TNF-α in the serum of old mice support this view. Furthermore, the in vivo data may not be directly comparable to in vitro studies that used isolated peritoneal macrophages because of functional differences determined by the tissue from which they were isolated and the method of isolation.

Several B and T lymphocyte functions, such as the ability to produce antibodies in response to antigen stimulation, mitogen-induced proliferation, and cell-mediated immune responses, also decrease with advancing age. The impairment of cell-mediated immune reactivity associated with aging seems to be due mainly to a defect in the production of IL-2 by helper T cells, but the impaired capacity of macrophages to produce IL-1 in vitro and the reduced binding of cytokines by T cells could also serve as contributing factors. It is important to note, however, that the phagocytic activity of monocytes and macrophages in aged mice and rats is normal. The phagocytic function of blood monocytes in elderly persons is similar to that in young persons. Interestingly, phagocytic activity, lysosomal acid phosphatase activity, DNase activity, and the synthesis of colony-stimulating factor in cultured macrophages were significantly increased in 17-month-old mice compared with 2-6-month-old, or young, mice. The age-dependent increase in macrophage activity is particularly pronounced in New Zealand black mice, which exhibit progressively depressed cellular immunity. Interestingly, impaired T-cell function is also associated with hypertension, a known risk factor for stroke. Moreover, the number of activated monocytes is significantly elevated in mature hypertensive rats compared with normotensive rats. Thus, it is possible that the decreased cellular immunity in aging and hypertension is compensated for by an increase in the number and activity of macrophages, as reported in athymic nude mice.

The exaggerated production of TNF-α in vivo has been demonstrated in spontaneously hypertensive rats. Expression of RNA message for monocyte chemoattractant protein-1 and immunoreactive TNF-α has also been detected in human atherosclerotic lesions. Our recent studies demonstrated increased expression of the intercellular adhesion molecule (ICAM)-1, increased accumulation of macrophages, and increased in vitro production of TNF-α by carotid arteries of hypertensive rats. Preliminary studies demonstrated enhanced in vitro production of TNF-α in carotid arteries obtained from aged Sprague-Dawley rats. The aged rats have also been reported to exhibit an increased reactivity to TNF-α or IL-1 in that the cytokine-induced increase in the plasma levels of plasminogen activator inhibitor activity in aged rats was greater than in young rats.

According to our working hypothesis, the relevance of the increased cytokine release to stroke lies in the ability of cytokines such as TNF-α to prepare vessels for ischemic inflammatory tissue damage through increased adhesion of leukocytes and platelets on vascular endothelium and transformation of the endothelial surface from an actively anticoagulant to a procoagulant state. Some of the specific endothelial changes under the influence of TNF-α include the synthesis and surface expression of tissue factor, the synthesis and expression of PAF, the release of IL-1, the release of factor VIII, and the enhanced adhesion of monocytes and granulocytes through the expression of adhesion receptor molecules. In addition, TNF-α can inhibit anticoagulant mechanisms such as the thrombomodulin--protein C--protein S system and decrease fibrinolysis as the levels of plasminogen activator inhibitor are increased. The overall effect of these interactions is to render the endothelial cell surface procoagulant, increase the adhesion of platelets and leukocytes, and increase local permeability. Our working hypothesis thus suggests that an interaction between perivascular macrophages and endothelium through cytokines could contribute to an increased risk of stroke in advanced age. We believe that our findings could be potentially relevant to human stroke in that the altered state of blood vessels induced by stroke risk factors could render such vessels locally vulnerable to thrombosis or hemorrhage in response to transient activation of intravascular coagulation or of complement by any of a wide variety of mechanisms (factors such as natural oscillation of coagulation potential, stress, infection, trauma, and inflammation). The site of thrombus formation would be determined by the preexisting vessel disturbance and...
not by the intravascular process activating the complement or coagulation systems, thereby following the local Shwartzman reaction paradigm. In this view, stroke would develop as the focal consequence of disturbed regulation of systems mediating coagulation, inflammation, and immunity, which are normally required for preservation of the integrity of the organism. Increased risk for a thromboembolic stroke could be possible if this process were to take place mainly in the extracranial vessels supplying the brain, whereas changes occurring in the intraparenchymal microvessels could contribute to lacunar strokes. Although our present data seem to support the working hypothesis, it should be emphasized that the true relevance of our findings to human stroke can be assessed only in forthcoming clinical studies that examine the role of the cytokine system in stroke patients and individuals with stroke risk factors.

The levels of TNF-α in the CSF were greater after the intracerebroventricular injection of lipopolysaccharide than after its systemic administration, indicating a local production of TNF-α within the central nervous system. These findings agree with earlier studies, which showed that the TNF-α activity was greater in the CSF than in blood after the intracerebroventricular injection of lipopolysaccharide in hypertensive rats. The perivascular macrophages derived from bone marrow could account for the production of TNF-α in the brain, because monocytes and macrophages are a major source for TNF-α. However, several other cell types in the central nervous system are capable of TNF-α expression. The ameboid microglia share many features with macrophages, including the production of IL-1 and TNF-α. Astrocytes may also be capable of transforming into macrophage-like cells and have been shown to release cytokines in vitro. Furthermore, macrophages within ependyma and meninges could also contribute to the TNF-α release into the CSF. In a previous study, we found that lipopolysaccharide induced a concentration-dependent TNF-α release in isolated rat brain slices. The level of TNF-α reached 3,000 ± 263 units/mg protein per hour after incubation with lipopolysaccharide (1 mg/mL). Because the meninges were routinely removed before slices were prepared for incubation, a predominant contribution by meningeal macrophages to the TNF-α release in the brain slices seems unlikely. In a recent study, TNF-α mRNA has been reported to be expressed in the normal mouse brain, but the effect of lipopolysaccharide was not tested. Surprisingly, the predominant cells expressing TNF-α mRNA in the mouse brain were neurons, although expression was also seen in ependymal cells and, to a lesser degree, in microglia. However, the TNF-α message in the brain cells was not translated to the protein product because the cells positive for TNF-α mRNA did not contain detectable levels of immunoreactive TNF-α. Thus, the relative contribution of the various cell types to the release of TNF-α into CSF in intact animals will not be clarified until studies using in situ hybridization and immunohistochemistry to reveal the exact cell types expressing mRNA for TNF-α after lipopolysaccharide stimulation in vivo become available. The TNF-α released into the CSF could contribute to the ischemic and hemorrhagic lesions found in the brain stem of aged rats after a provocative dose of lipopolysaccharide. This inference is supported by the recent finding of hemorrhagic necrosis of the neuropil after the administration of recombinant TNF-α into the central nervous system.

The present study also demonstrated an exaggerated release of an immediate proinflammatory mediator in the brains of aged animals, as evidenced by the increase of PAF in the CSF after the intracerebroventricular administration of provocative doses of lipopolysaccharide. PAF is a lipid mediator that induces platelet aggregation, increases vascular permeability, and acts as an endothelial adhesion receptor for white blood cells. PAF in the CSF could originate from multiple cell types, including the endothelial cells, monocytes and macrophages, neutrophils, glial cells, and neurons. However, the mononuclear cells have been proposed as the primary source for PAF in responses to lipopolysaccharide. Interestingly, TNF-α can induce PAF release from endothelial cultures. This finding may bear relevance to our results, because the high levels of PAF and TNF-α found in the CSF after lipopolysaccharide administration were temporally related.

In conclusion, the present study provides support for our working hypothesis that advanced age, a risk factor for stroke, creates a state of increased probability of an interaction between monocytes/macrophages and endothelial cells, thereby enhancing local thrombosis or hemorrhage through increased activity of cytokines. According to this hypothesis, risk factors for stroke are initially associated with a change in endothelium and a hyperactive state of the monocytes/macrophages. The endothelial change would probably involve the expression of endothelial adhesion receptors for monocytes, which in turn would facilitate the adherence of monocytes to the vessel wall and transendothelial migration in both large and small vessels, leading to local deposits of monocyte clusters in segments of the blood vessels. These monocyte clusters could then periodically signal the vessel endothelium through the release of prothrombotic, proinflammatory, and chemotactic mediators such as TNF-α, PAF, and, perhaps, IL-1 to convert the endothelium to a procoagulant state and, in effect, prepare those vessel segments in a manner similar to the localized Shwartzman paradigm. Complement activation or any stimulus leading to activation of the coagulation system (factors such as natural oscillation of coagulation potential, stress, infection, trauma, and inflammation) could then precipitate a localized reaction within this prepared vessel segment and lead to a local thrombosis or hemorrhage. Because we have chosen to test this hypothesis first in an animal model, the conclusions of this study at this point are restricted to the species used. However, many aspects of this work can be tested in humans, and it remains for these future studies to show whether the proposed mechanisms are operating in clinical stroke.

Acknowledgments

The authors wish to thank Dr. Stephanie Vogel, Department of Microbiology, Uniformed Services University of the Health Sciences, for the generous supply of L-929 cells; Ms. Monika Grojec and Mr. James H. Millson for excellent technical assistance; and Ms. B.J. Hessie for skillful editing.

References

Increased release of tumor necrosis factor-alpha into the cerebrospinal fluid and peripheral circulation of aged rats.
A L Sirén, Y Liu, G Feuerstein and J M Hallenbeck

Stroke. 1993;24:880-886
doi: 10.1161/01.STR.24.6.880

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/24/6/880