recovery of mechanical function and improved adenosine triphosphate regeneration during reperfusion.[11]

In initiating preconditioning with noxious stimuli such as toxins, stress, or ischemia, an optimum that constrains therapeutic applicability is to be expected. Another drawback of using lipopolysaccharide (or its derivatives) for inducing cardiac protection is the relatively long-time scale required. Preconditioning achieved directly by clinically acceptable α_1-agonists bypasses the need for ischemic stress and is achieved within minutes. However, in patients most likely to benefit from preconditioning (e.g., before elective bypass I/R), the myocardium is diseased and perhaps intolerant to ischemia or excessive sympathetic stimulation. Also, α_1-adrenergic mechanisms of protecting postischemic function are short-lived (a few hours) and probably do not involve protection from antioxidants or stress protein synthesis, which lasts for days. These two different protocols may be refined by further mechanistic delineation, keeping desirable features while discarding elements such as inflammation, impaired respiration, or α_1-adrenergic vasoconstriction. Moreover, these and other strategies may be combined to allow full use of yet unknown endogenous defenses that may have evolved in various tissues against I/R injury.

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PERIVASCULAR MONOCYTE/MACROPHAGE INTERACTION WITH ENDOTHELIUM AS A MECHANISM THROUGH WHICH STROKE-RISK FACTORS OPERATE TO INCREASE STROKE LIKELIHOOD

Hypertension, a well-documented risk factor for stroke, is associated with atherosclerosis, but the precise mechanism by which it predisposes the patient to stroke remains uncertain. We are interested in the possibility that an interaction between perivascular macrophages and endothelium through cytokines could underlie this predisposition. Increased numbers of macrophages have been observed around large arteries in hypertensive or old rats.[1] These cells also appear in large numbers in a perivascular location in hyperlipidemia and diabetes mellitus[2] and are considered one of the earliest participants in the evolution of atherosclerotic lesions.[3] The RNA message for monocyte chemoattractant protein-1, a molecule with powerful monocyte chemoattractant activity expressed by monocytes, endothelial cells, and smooth muscle cells, and immunoreactive tumor-necrosis factor-α (TNFα) have been detected recently in atherosclerotic lesions.[4,5] Thus it appears that many elements of the putative macrophage-endothelium interaction are in place in the diseased vascular segments of animals with various stroke risk factors, including hypertension.

We are also intrigued by the possibility of a relationship between the localized Shwartzman phenomenon[6] and acute stroke. This phenomenon was initially described in 1928 during efforts to develop a vaccine for typhoid fever.[6] When the endotoxin lipopolysaccharide, an extract from *Bacillus typhosus* cultures, was injected into a rabbit's skin, the area would redden but the erythema would disappear in 24 to 48 hours if nothing further was done.[7] When the endotoxin was injected into an ear vein, there was no visible response unless the animal had received an intradermal injection of endotoxin some 18 to 24 hours before the intravenous injection. In this special case, all tissues of the body that had not been "prepared" by the initial intradermal injection seemed unaffected by the "provocative" intravenous injection. The site of the preparatory intradermal injection, however, showed a dramatic change. The initial zone of redness covering an area 15 to 20 mm in diameter would enlarge to approximately 30 to 50 mm in diameter during the course of several hours. Thrombosis and hemorrhage in dermal vessels caused the change in appearance. In recent years it has become clear that exposure of vessels to TNFα and interleukin-1 (IL-1) is a sufficient stimulus for preparation, and a sufficient provocative stimulus involves activation of coagulation (Hageman

factor, factor XII) or inflammation (complement),[8] so the localized Shwartzman reaction is a general phenomenon and not restricted to lipopolysaccharide mediation.

If a mechanism similar to the local Shwartzman phenomenon were to operate in the genesis of stroke, a vessel segment periodically exposed to effective levels of the cytokines TNFα and IL-1 would be brought into a state in which its endothelium would be activated,[9] and the vessel segment could be considered prepared and ready to be triggered for a period of hours. If, during this critical interval, the coagulation or complement system should become activated, the prepared vessel segment could undergo thrombosis or a sequence of events leading to hemorrhage.

Our first series of experiments addressed the issue of whether various risk factors for stroke effectively prepared brain stem vasculature of rats for a localized Shwartzman reaction.[10] Lipopolysaccharide was injected intracisternally or intravenously into rats with and without identifiable stroke risk factors, and the incidence of brain stem stroke was quantified. Rats with hypertension, hypertension plus genetic stroke proneness, advanced age, or streptozocin-induced "diabetes" reacted with a significantly higher incidence of stroke than did rats devoid of such stroke risk factors.

For further analysis, we generated a working hypothesis that hypertension creates a state in which there is an increased probability of an interaction between monocytes or macrophages and cerebrovascular endothelial cells that is mediated by TNFα and IL-1 and could lead to thrombosis or hemorrhage in focal regions of brain vasculature. A series of studies has been carried out that addresses various elements of this hypothesis. The expression of intercellular adhesion molecule-1 (ICAM-1) in the carotid artery and brain intraparenchymal vessels and the accumulation of monocytes and macrophages in the same vessels were compared in hypertensive rats (SHR), hypertensive and stroke-prone rats (SHR-SP), and rats devoid of risk factors for stroke (WKY). Perfusion-fixed frozen sections (16 μm) of carotid arteries were exposed to specific monoclonal antibodies against rat ICAM-1 (IA29) or rat monocyte/macrophages (ED-1). Brain sections (16 μm) from these same rat strains were exposed to IA29 or ED-2, a monoclonal antibody specific for rat macrophages. All sections were double stained with antifactor VIII antibodies for the identification of endothelial cells. Hypertensive animals, but not normotensive WKY rats, were found to express ICAM-1 on their carotid arteries and to have identifiable cells of the monocyte/macrophage line adherent to the endothelium of these vessels and in a subendothelial location. Hypertensive rats also had larger numbers of macrophages in a perivascular location than in their normotensive counterparts. The number of perivascular macrophages per square millimeter of high power field detected by ED-2 immunostaining was significantly greater in SHR-SP and SHR than in WKY rats.

Carotid rings (2 mm) from SHR and WKY rats were incubated in a 24-well tissue culture dish with either lipopolysaccharide (100 ng/ml) or sterile saline solution (20 μl) for 8 hours. The TNFα content of the medium was measured by means of the L 929 cell bioassay. The SHR carotid artery rings produced more TNFα than did WKY rings (313 ± 62 and 91 ± 15 units/mg wet weight, respectively; $p < 0.05$; $n = 16$).

Similar studies were done in 16-week-old and 2-year-old Sprague Dawley rats (SD_{16w} and SD_{2y}, respectively) to investigate the putative interaction. Brain sections exposed to ED-2 revealed greater numbers of perivascular macrophages in SD_{2y} (7.5 ± 0.9; $n = 9$) than in SD_{16w} rats (3.7 ± 1.2; $n = 4$) ($p < 0.05$). The SD_{2y} carotid artery rings exposed to lipopolysaccharide produced more TNFα than did SD_{16w} rings (533 ± 127 and 219 ± 90 units/mg wet weight, respectively; $p < 0.05$; $n = 8$).

The data, in aggregate, extend documentation that the elements of the putative interaction between monocytes or macrophages and endothelium are in place in rats with the stroke risk factor of hypertension and, in general, they function according to the working hypothesis. Further studies are needed to clarify important details and establish whether the putative interaction is causally related to stroke.

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Supported in part by PHS grant NS 28225

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