Interacting Leukocytes Predict Atherosclerosis and Restenosis

To the Editor:

Previous studies have revealed the association between inflammation, atherosclerosis and cardiovascular diseases (CVD). Several recent investigations have addressed the involvement of pathological intimal thickening after macrophage infiltration and circulating apoptotic progenitor cells with telomere repeat-binding factor TRF2 in early atherosclerosis and prediction of carotid recurrent stenosis using intraplaque hemorrhage. The ultimate mechanism of atherogenesis and recurrent stenosis remains unclarified. In a previous study, we demonstrated vascular remodeling with inflammatory cells (monocytes/macrophages) infiltration in cerebral arteries of hypertensive rats. Our study attempted to elucidate the vascular changes leading to atherosclerosis, and to propose the predictive factor for restenosis. Five-week-old male spontaneously hypertensive rats (SHR) were obtained from National Animal Center. They were fed with standard rat chow and tap water, and kept in animal room with adequate environmental control. The tail cuff pressure (TCP) represents the systolic pressure which was detected using a photoelectric volume oscillometer (UR-5000, Ueda). At 9 weeks, euthanasia was done with a high-dose of intraperitoneal pentobarbital (100 mg/kg). They were fixed by transcardial perfusion with 4% paraformaldehyde. The internal carotid arteries (ICA) were excised. The specimens were then dehydrated, embedded in paraffin, sectioned into 5-μm thickness. For immunohistochemical ED1 stain and periodic acid-Schiff’s reaction (PAS) examinations, the sections were deparaffinized in xylene and ethanols and rehydrated 3 times for 5 minutes in solution containing 0.02% Triton X-100 plus 0.01% bovine serum albumin. Sections were preincubated with 2% horse serum to minimize nonspecific binding. They were incubated overnight at 4°C with mouse antirat macrophage/monocyte antibody (anti-ED1, Serotec). The slides were incubated with biotinylated rabbit antimouse IgG as secondary antibody. The bound primary antibody was visualized using avidin-biotin-peroxidase method (ABC Elite Kit; Vector Laboratories).

TCP was elevated in a time-dependent fashion to an average of 171 mm Hg (95% CI: 160 to 181 mm Hg) at 9 weeks. Hypertension caused various morphological changes in ICA. The PAS staining micrograph showed inflammatory cells infiltration in endothelial layer (Figure, A). The positive ED1 cells (monocytes/macrophage) exist in and on endothelial layers, and intraluminal aspect of endothelial layer in ICA of SHR. The positive ED1 cells (monocytes/macrophage) existed in endothelial (white arrow), on endothelial (white arrowheads) layers and intraluminal aspect of endothelial layer (black arrowhead) of ICA in SHR. The interacting leukocytes adhered to intraluminal aspect of endothelial layer possess interacting materials, which may be TRF2. In the ED1 staining micrograph (Figure, B), our study also displayed the character of interacting leukocytes without adhesive plaque in the inflammatory vessels in PAS stain (Figure).

Hypertension induced various morphological changes in ICA of SHR. These structural alterations may be the initial events leading to the early development of atherosclerosis. Our study also revealed that hypertension (TCP >160 mm Hg) for 2 weeks promoted the genesis of atherosclerosis. The risk degrees and duration of inflammation are important to the formation of CVD or stroke. The interacting leukocytes which appear in the vascular intima and lumen may be used as predictive biomarker for atherosclerosis and restenosis after treating CVD. The causes of inflammation may be the stimuli. We also propose advanced investigations to determine whether the interacting leukocytes are TH17 cells with circulating apoptotic progenitor cells (lipoprotein) in ED1 stain are TRF 2 in whole or in a part.
Acknowledgments
The authors are grateful to A. Huang for the immunohistochemical staining.

Sources of Funding
This study was supported in part by the Committee for Research in Tao-Yuan General Hospital and by a grant (PTH 9608) from the Institute for Research in Tao-Yuan General Hospital.

Disclosures
None.

Nan-Kuang Hsieh, MD, PhD, FICS
Department of Family Medicine
Tao-Yuan General Hospital
Department of Leprosy Control and Prevention
Lo-Sheng Leprosarium
Department of Health
The Executive Yuan
Taoyuan, Taiwan

Hsing I. Chen, MD, PhD
Institute of Integrative Physiology and Clinical Sciences
Tzu Chi University
Hualien, Taiwan

10. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med. 2007;13:139–145.
Interacting Leukocytes Predict Atherosclerosis and Restenosis
Nan-Kuang Hsieh and Hsing I. Chen

Stroke. 2007;38:e162-e163; originally published online November 1, 2007;
doi: 10.1161/STROKEAHA.107.496455
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

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/38/12/e162