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Monocytic adhesion molecule expression and monocyte–endothelial cell dysfunction are increased in patients with peripheral vascular disease *versus* patients with abdominal aortic aneurysms

Eugene S. Lee, MD, PhD,[a,b,*] *Elyse N. Van Spyk, BS,*[b] *Kevin C. Chun, BS,*[b] *Robert L. Pitts, BS,*[a] *Mack H. Wu, MD,*[c] *and Sarah Y. Yuan, MD, PhD*[d]

[a] *Department of Surgery, University of California, Davis, Sacramento, California*
[b] *Department of Surgery, Sacramento VA Medical Center, Mather, California*
[c] *Department of Surgery, Morsani College of Medicine, University of South Florida, Tampa, Florida*
[d] *Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, Florida*

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ABSTRACT

Background: Statin therapy is used in the medical management of patients with peripheral vascular disease (PVD) and abdominal aortic aneurysm (AAA) for the pleiotropic and anti-inflammatory benefits. We hypothesize that the inflammatory mechanisms of monocyte–endothelial cell interactions in endothelial barrier dysfunction are more significant in patients with PVD compared with those with AAA. The purpose of this study was to assess patient peripheral blood monocyte adhesion molecules by flow cytometry and monocyte-induced endothelial barrier dysfunction by using an *in vitro* endothelial cell layer and electric cell-substrate impedance sensing (ECIS) system.

Methods: Peripheral blood was collected from patients with either PVD (ankle-brachial index <0.9, toe–arm index <0.8, or required lower extremity vascular intervention) or AAA (aortic diameter >3.0 cm). Monocytes were isolated from fresh whole blood using an accuspin-histopaque technique. The separated monocytes underwent flow cytometry analysis to evaluate the expression levels of the cell membrane adhesion molecules: CD18, CD11a/b/c, and very late antigen-4. Endothelial cell function was assessed by adding monocytes to an endothelial monolayer on ECIS arrays and coculturing overnight. Peak changes in trans-endothelial electrical resistance were measured and compared between patient groups.

Results: Twenty-eight monocyte samples were analyzed for adhesion molecules (PVD, 19 and AAA, 9) via flow cytometry, and 11 patients were evaluated for endothelial dysfunction (PVD, 7 and AAA, 4) via ECIS. There was no significant difference between risk factors among PVD and AAA patients except for age, where AAA patients were significantly older than PVD patients in both flow cytometry and ECIS groups ($P = 0.02$ and 0.01, respectively). There were significantly higher levels of adhesion molecules CD11a, CD18, and CD11c (averaged mean fluorescent intensity P values: 0.047, 0.038, and 0.014, respectively) in PVD patients compared with AAA patients. No significant difference was found for CD11b and

* *Corresponding author*. 4860 Y Street, Suite #3400, Sacramento, CA 95817. Tel.: +1 916 843 7202; fax: +1 916 734 7205.
E-mail address: EugeneS.Lee@ucdmc.ucdavis.edu (E.S. Lee).
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very late antigen-4 expression ($P = 0.21$ and 0.15, respectively). There was significantly more monocyte–endothelial cell dysfunction in patients with PVD *versus* patients with AAA, with a maximal effect seen at 15 h after monocyte addition ($P = 0.032$).

Conclusions: Patients with PVD have increased expression levels of certain monocyte adhesion molecules and greater monocyte-induced endothelial layer dysfunction compared with those with AAA. This may lead to other methods of targeted therapy to improve outcomes of these vascular patients.

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1. Introduction

Statin therapy has long been established as beneficial for nonsurgical populations by lowering cholesterol levels to prevent adverse cardiovascular outcomes [1]. Increasingly, secondary effects of the benefits of statin therapy, other than cholesterol lowering, have been realized [2]. Favorable vascular remodeling in the carotid arteries [3] and improved angiographic appearance of coronary arteries [4] are just a few of the determined "pleiotropic effects" of statin therapy. More recently, statins administered to patients undergoing noncardiac vascular surgery have shown a significant decrease in myocardial ischemia and death from cardiovascular causes [5].

Biologically, statins have been hypothesized to have several plaque-stabilizing effects, such as increasing the expression of endothelial nitric oxide synthase, decreasing the thrombogenic profile, and reducing inflammation through extravasion of monocytic macrophages [6]. These proposed mechanisms support the theory that atherosclerosis is an inflammatory process [7].

In determining monocytic interaction and inflammation in vascular surgical patients, we sought to evaluate two models of vascular disease that share similar yet distinct pathogenesis. Although inflammation plays an integral role in both disease states, abdominal aortic aneurysm (AAA) patients have a distinct long-term prognosis and survival. AAA patients are reported to have an estimated 11-y life expectancy after diagnosis [8], whereas peripheral vascular disease (PVD) patients can have a lower life expectancy of 6 mo to around 5 y [9]. Recent evidence suggests that AAA patients may live longer because of better clinical management of risk factors, including an increase in statin use and a decrease in tobacco use in those aged >65 y [8,10,11]. We hypothesize that the cellular mechanisms of monocyte–endothelium interactions associated with vessel barrier dysfunction are more severe in patients with PVD compared with AAA because of increased monocyte adhesion molecule expression. The purpose of this study was to compare AAA and PVD patients' peripheral monocyte adhesion molecule expression via flow cytometry and monocyte-induced endothelial barrier dysfunction *in vitro*.

2. Materials and methods

2.1. Human study methods

A prospective evaluation of AAA and PVD patients' whole blood that underwent monocyte isolation, flow cytometry, and electric cell-substrate impedance sensing (ECIS) analysis was performed under Institutional Review Board approval at the Veterans Affairs Northern California Health Care System. All patients were enrolled and monitored at the Sacramento Veterans Affairs Medical Center.

2.2. Clinical data and risk factors

A patient history and physical examination were performed, and pertinent medical history was documented. Diagnosis, surgical procedure, age, aneurysm diameter in centimeter (for AAA patients), ankle-brachial index (ABI) score (for PVD patients), serum white blood cell in thousands per cubic millimeter, total cholesterol, statin use, and history of diabetes mellitus, hypertension, coronary artery disease, cerebral vascular accident, chronic obstructive pulmonary disease, cardiovascular disease, and tobacco use were recorded. PVD patients were defined as having ABIs of <0.9, had a significant drop in ABIs (>0.15) after treadmill exercise, a toe–arm index of <0.8 for patients who had noncompressive vessels, or patients who required documented vascular intervention to improve peripheral blood flow. AAA patients were defined as having an abdominal aortic diameter of >3.0 cm.

2.3. Monocyte isolation from human adult peripheral blood

After consent was obtained, 20 mL of blood was collected from patients during surgical intervention for AAA or PVD. The blood was immediately placed in four 5-mL polypropylene heparinized tubes and kept on a rocker until processed. In all instances, the blood was used within 2 h of removal from the patients. Monocytes were isolated as described by Sun *et al.* [12] with slight modification to the protocol described. Briefly, 4 mL of blood was overlaid over 4 mL of Ficoll–Hypaque density gradient (1.077 g/mL; BD Biosciences, Bedford, MA) in 4- to 15-mL Falcon tubes (BD Biosciences) and centrifuged for 30 min at 400 *g*. After separation, the buffy white layer of mononuclear cells was transferred via pipette into two sterile 15-mL Falcon tubes and rinsed with Hanks buffered salt solution (Sigma, St. Louis, MO) and then centrifuged at 250 *g* for 10 min. After aspiration, the pellet was resuspended in 2 mL red blood cell lysis buffer (Boston Bioproducts, Boston, MI) and allowed to sit for 5 min. Two milliliters of phosphate-buffered saline (PBS; pH 7.4) wash buffer solution (containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) [EMD chemicals, Cincinnati, OH] and 2% bovine serum albumin [US Biochem Corp, Cleveland, OH]) were then added, and the cell suspension was centrifuged at 250 *g* for 10 min. After aspiration, the pellet was resuspended in 500 μL of the PBS wash buffer and an EasySep monocyte isolation kit (Stem Cell,