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Monocyte activity is linked with abdominal aortic aneurysm diameter

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ABSTRACT

Background: Systemic inflammation and increased matrix metalloproteinase (MMP) cause elastin degradation leading to abdominal aortic aneurysm (AAA) expansion. Several prospective studies report that statin therapy can reduce AAA expansion through anti-inflammation. We hypothesize that monocyte activity plays a pivotal role in this AAA development and this study examines patient peripheral blood monocyte cell adhesion, transendothelial migration, and MMP concentrations between AAA and non-AAA patients.
Materials and methods: Peripheral blood was collected and monocytes isolated from control ($n = 15$) and AAA ($n = 13$) patients. Monocyte adhesion, transmigration, and permeability assays were assessed. Luminex assays determined MMP-9 and tissue inhibitor of metalloproteinase-4 (TIMP-4) concentrations from cell culture supernatant and patient serum.
Results: AAA patient monocytes showed increased adhesion to the endothelium relative fluorescence units (RFU, 0.33 ± 0.17) *versus* controls (RFU, 0.13 ± 0.04; $P = 0.005$). Monocyte transmigration was also increased in AAA patients (RFU, 0.33 ± 0.11) compared with controls (RFU, 0.25 ± 0.04, $P = 0.01$). Greater numbers of adhesive ($R^2 = 0.66$) and transmigratory ($R^2 = 0.86$) monocytes were directly proportional to the AAA diameter. Significantly higher serum levels of MMP-9 (2149.14 ± 947 pg/mL) were found in AAA patients compared with controls (1189.2 ± 293; $P = 0.01$). TIMP-4 concentrations were significantly lower in AAA patients (826.7 ± 100 pg/mL) compared with controls (1233 ± 222 pg/mL; $P = 0.02$). Cell culture supernatant concentrations of MMP and TIMP from cocultures were higher than monocyte-only cultures.
Conclusions: Monocytes from AAA patients have greater adhesion and transmigration through the endothelium *in vitro*, leading to elevated MMP-9 levels and the appropriate decrease in TIMP-4 levels. The ability to modulate monocyte activity may lead to novel medical therapies to decrease AAA expansion.

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1. Introduction

Abdominal Aortic Aneurysm (AAA) rupture is a significant cause of death among elderly white males in the United States today. Studies have found ultrasound screening for AAA as an effective method to prevent aneurysm-related deaths and reduce all-cause mortality [1–4]. AAA is diagnosed when the maximum aortic diameter is ≥3.0 cm, and an aortic diameter ≥5.5 cm is generally considered for surgical repair [5]. However, the management of ectatic and small abdominal aortic aneurysms (2.5–5.4 cm) remains a challenge. For instance, clinicians have recommended follow-up intervals to be from 6 mo to 2 y, but a meta-analysis has shown that follow-up can be extended to 7.4 y because these AAAs expand slowly [6]. Whether these recommendations can safely applied to clinical practice has yet to be explored, but more importantly, a better understanding of the mechanism of AAA expansion should also be studied.

AAA development is a product of the extracellular matrix (ECM) breakdown resulting from genetic abnormalities, hemodynamic shear stress, and inflammation [7,8]. Monocytes, circulating blood leukocytes that play an important role in the inflammatory response, have been implicated in the process of vascular remodeling and AAA expansion [9,10]. Monocyte activity during inflammation is modulated by a variety of mediators such as cytokines, chemokines, and growth factors that allow interaction with the endothelium of the vessel wall through the upregulation of adhesion molecules. During this process, proinflammatory cytokines such as tumor necrosis factor α and interleukin 1β promote the secretion of matrix metalloproteinase (MMP) via the mitogen-activated protein kinase pathways [11]. MMPs are zinc-based proteases responsible for remodeling the ECM and play an important role in tissue development and homeostasis [12]. Endothelial cells are bound to the ECM by focal adhesions, composed of integrin transmembrane and actin-linking proteins to withstand fluid shear stress and maintain endothelial barrier function [13]. MMPs degrade ECM components by allowing smooth muscle cells and other circulating cells to migrate through the endothelial barrier [14]. This action plays a large part in regulating endothelial permeability. Deregulation can cause hyperpermeability that allows additional activated monocytes to migrate through the vessel wall (Fig. 1). Although endothelial permeability is essential for wound healing, hyperpermeability exacerbates endothelial injury by allowing foreign cells such as smooth muscle cells and ECM components to infiltrate the lumen of the vessel.

We believe that the patient peripheral blood monocytes are a key factor in inciting AAA formation and expansion through inflammation and upregulation of MMPs. Multiple clinical trials have shown statin therapies and anti-inflammatories prevent aneurysm expansion [15,16]. However, the mechanism by which statins prevent AAA expansion has yet to be elucidated. We hypothesize that patient monocytes interacting with the aortic endothelium via adhesion and transmigration leads to endothelial cell hyperpermeability, ECM destabilization, and subsequent AAA expansion. The purpose of this study is to identify the role of monocyte activity and the production of MMP in the regulation of vascular inflammation and AAA expansion.

2. Materials and methods

2.1. Ethical approval

All patients for this study were recruited at the Sacramento Veterans Affairs (VA) Medical Center in Mather, CA under an approved VA Northern California Health Care System institutional review board protocol.

2.2. Clinical data and risk factors

Patients selected were male volunteers from the VA Vascular Clinic who presented with a minimum AAA diameter of 3.0 cm. Controls were age-matched male volunteers also recruited from the VA Vascular clinic with no incidence of an AAA. A patient history and physical examination was performed for each participating subject. A maximum AAA diameter was determined from abdominal ultrasonography or computerized tomography scan. The results were verified by a radiologist or a vascular specialist and recorded into the patient's electronic medical record. The risk factors obtained from the patient's electronic medical record were age, hypertension, diabetes, current smoking, statin use, total cholesterol (mg/dL), body mass index (kg/m^2), estimated glomerular filtration rate (mL/min), and the presence of coronary artery disease, peripheral vascular disease, chronic obstructive pulmonary disease, and cerebral vascular accident.

2.3. Monocyte isolation

Twenty milliliters of peripheral blood was taken from consented control subjects and AAA patients. The blood was directly placed in four 5-mL polypropylene heparinized tubes (BD Biosciences, Bedford, MA) and kept on a rocker until processed (within 2 h of blood retrieval). Monocytes were isolated as described by Sun *et al.* [17] with slight modification. Briefly, an equal amount of blood was overlaid over Ficoll Histopaque density gradient (1.077 g/mL; BD Biosciences) in Falcon tubes (BD) and centrifuged for 30 min at 400*g*. The peripheral blood mononuclear cells were isolated and rinsed with equal parts Hanks balanced salt solution (Sigma, St. Louis, MO). After centrifugation and aspiration, the pellet was resuspended in 2 mL red blood cell lysis buffer (Boston Bioproducts, Boston, MI) and allowed to sit protected from light for 5 min. Two milliliters of phosphate buffered saline (PBS) (pH 7.4) wash buffer solution (containing 0.5 mM ethylenediaminetetraacetic acid [EMD chemicals, Cincinnati, OH]) and 2% bovine serum albumin (US Biochem. Corp, Cleveland, OH) were added to the sample and centrifuged at 250*g* for 10 min. After aspiration, the pellet was resuspended in 500 μL of the PBS wash buffer. An EasySep monocyte isolation kit (StemCell, Vancouver, British Columbia, Canada) was used to enumerate and