ELSEVIER

Atherosclerosis 194 (2007) e9–e17

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Oxidized LDL specifically promotes the initiation of monocyte invasion during transendothelial migration with upregulated PECAM-1 and downregulated VE-cadherin on endothelial junctions

Ken Hashimoto [a,*], Noriyuki Kataoka [b], Emi Nakamura [a], Katsuhiko Tsujioka [a], Fumihiko Kajiya [b]

[a] *Department of Physiology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan*
[b] *Department of Medical Engineering, Kawasaki Medical School, Japan*

Received 26 April 2006; received in revised form 6 November 2006; accepted 21 November 2006
Available online 27 December 2006

Abstract

It is poorly understood how oxidized LDL (oxLDL) promotes monocyte dynamics in transendothelial migration (TEM) in atherogenesis. We developed an *in vitro* 3D-live-single cell TEM assay system with subendothelial oxLDL embedded in ultra-thin collagen gels, mimicking subendothelial oxLDL accumulation *in vivo*. With dividing monocyte dynamics into three stages (1: adhesion on endothelium, 2: invasion and 3: complete transmigration below endothelium), we analyzed the stage transition dynamics of individual living human monocytes. OxLDL did not enhance initial monocyte adhesion to endothelium (stage 1), but it specifically primed adherent monocytes to start invasion (stage 1 → 2). Once invasion started, it had no effect thereafter on monocyte stage transition (stage 2 → 3). OxLDL upregulated PECAM-1 and downregulated VE-cadherin on endothelial junctions without monocyte addition, both of which could promote monocyte entry by enhanced homophilic binding to monocyte PECAM-1, and by disrupted junctional barrier, respectively. Meanwhile, monocyte speed at neither locomotion on endothelium (stage 1) nor subendothelial migration (stage 3) was altered by oxLDL. These data indicate that before monocyte adhesion, endothelial junctions changed their conformation to more monocyte-acceptable state in response to oxLDL, resulting the stage-specific promotion of monocyte TEM (stage 1 → 2; initiation of invasion) with no enhancement of its initial adhesion or migration speed.

Keywords: Monocyte; Transendothelial migration; Endothelium; Oxidized LDL; PECAM-1; VE-cadherin; Atherosclerosis

1. Introduction

One of the crucial events in early atherosclerosis is the recruitment of blood monocytes and T-lymphocytes to proatherogenic vascular regions and subsequent transendothelial migration (TEM). This process involves a multi-step cascade between leukocyte and endothelial cell (EC) adhesion molecules including rolling, firm adhesion, locomotion and transmigration [1,2]. The importance of post-adhesion locomotion on endothelium has recently been reported [2]. As for paracellular transmigration, many involved molecules have been reported including platelet endothelial cell adhesion molecule-1 (PECAM-1) [3–5], vascular endothelial cadherin (VE-cadherin) [5,6], junctional adhesion molecules (JAMs) [7], inter-cellular adhesion molecule-2 (ICAM-2) [8] and CD99 [4]. PECAM-1 is recruited to the junctional zone of transmigrating leukocyte [3], which could enhance leukocyte traction with homophilic binding to leukocyte PECAM-1. VE-cadherin acts as a junctional barrier that is controlled by actomyosin-based contractility [9]. Previously we demonstrated that ECs softened mechanically with monocyte adhesion [10] with reduction of stress fibers and focal

* Corresponding author. Tel.: +81 86 462 1111x83512; fax: +81 86 464 1020.
E-mail address: khashimo@med.kawasaki-m.ac.jp (K. Hashimoto).

0021-9150/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2006.11.029

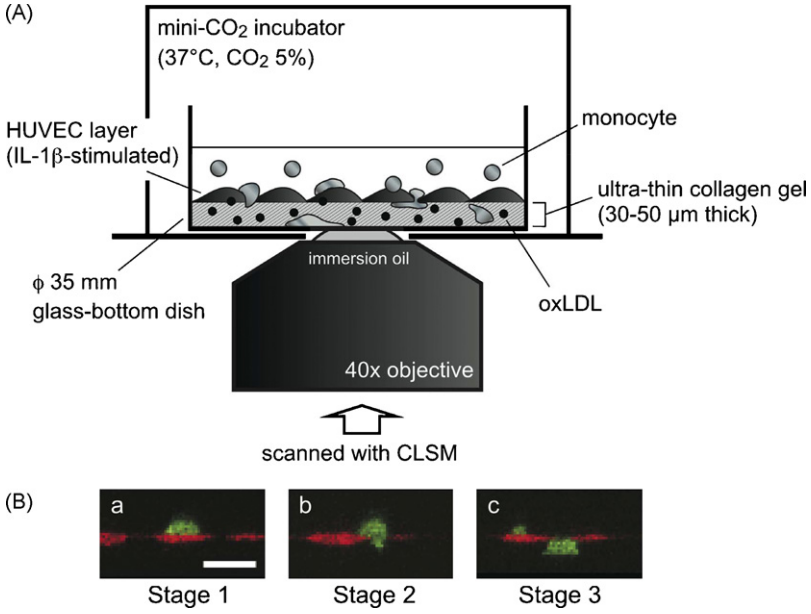

Fig. 1. (A) 3D-live-single-cell assay system for monocyte TEM. OxLDL was embedded in subendothelial collagen gels. Freshly-isolated, green-dye-stained human monocytes were added to IL-1β-stimulated, red-dye-stained HUVECs, and TEM dynamics was tracked three-dimensionally with CLSM equipped with mini-CO_2 incubator at 2–5 min intervals. Ultra-thin collagen gel layer (30–50 μm thick) on glass-bottom dish provides the best-suited thickness for both monocyte invasion and observation with high magnification (40×) under the inverted microscope. (B) Representative 3D-cross-sectional images at each stage of monocyte (green). Stage 1: adhesion on ECs (red), stage 2: invasion and stage 3: complete transmigration below ECs. Bar = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

adhesion kinase (p125FAK) [11]. Furthermore endothelial cell-to-substrate gaps immediately increased in nanometer order with monocyte adhesion [10]. These micro-mechanical analyses suggest that ECs weaken their adhesiveness to the substrate, and become fragile with monocyte adhesion, which could promote subsequent transmigration.

Oxidized LDL (oxLDL) has been reported to have broad effects on vascular cells to promote atherogenesis, including upregulation of vascular cell adhesion molecule-1 (VCAM-1), ICAM-1 [12,13] and monocyte chemotactic protein-1 (MCP-1) [14] on ECs. In the atherosclerotic lesions *in vivo*, oxLDL was reported to accumulate predominantly in subendothelium, possibly because relatively low level of antioxidants in subendothelium compared with those in plasma [15]. Many reports have shown that monocyte TEM is facilitated by oxLDL, most of which was analyzed with widely-used Boyden chamber method [13]. However, it is almost unclear how and at which stage oxLDL promotes monocyte dynamics during multi-step TEM process. Boyden chamber method cannot be used to address this issue, because in this method, transmigration is viewed simply transmigrated or not at the end of the assay in a certain period of time. Recently we have developed an *in vitro* TEM system using human monocytes and umbilical vein endothelial cells (HUVECs) cultured on ultra-thin (30–50 μm thick) subendothelial collagen gels [16]. This system can trace and quantitatively analyze monocyte dynamics three-dimensionally, at single-cell level with living cells (examples shown in Fig. 1B), with which cell morphology, size, migration distance, speed and migration stage of each monocyte at each time can be analyzed [16]. In the present study, we applied this system for developing a proatherogenic model with subendothelial oxLDL embedded in collagen gels, mimicking *in vivo* subendothelial accumulation of oxLDL, and analyzed the changes of 3D-dynamics of individual living monocyte with oxLDL, together with biochemical analysis of rearrangements of EC junctional molecules.

2. Methods

2.1. Cell culture

HUVECs were purchased from Kurabo, Japan, and cultured as described [16] with a slight modification. For experiments, cells (passage 3) were seeded at 2500 cells/cm^2 to grow to confluency. Human monocytes were freshly isolated from peripheral blood as described [16]. All protocols involving human subjects were approved by an Institutional Review Committee.

2.2. Preparation of oxLDL

Native LDL from healthy human plasma (200 μg/mL, Athens Research & Technology, GA) was oxidized by 24 h co-incubation with 10 μM $CuSO_4$ (Sigma, MO) at 37 °C. Oxidation was terminated by 40 μM butylated hydroxytoluene (BHT; Sigma). OxLDL preparations were sterilized through 0.45 μm filter, and used immediately within a few hours [17]. Oxidation of LDL was monitored by the amounts