

CD11c+ as an inflammatory biomarker and therapeutic target for atherogenesis

Contact Information

Scott I Simon PhD Professor of Biomedical Engineering GBSF Rm. 3313, Health Science Dr Office (530) 752-0299 sisimon@ucdavis.edu	Kate Marusina, Ph.D., MBA Manager, Research Facilitation and Industry Alliance, Clinical and Translational Science Center UC Davis School of Medicine TEL: (916)703-9177 CEL: (530)979-1522 EMAIL: kate.marusina@ucdmc.ucdavis.edu
--	---

Partnering goals

1. To develop reagents and assays to establish CD11c as a biomarker and therapeutic target in monocyte driven atherosclerotic disease.
2. Explore utility of targeted blocking of CD11c immune function with small molecules or antibodies to alter course of monocyte recruitment to insipient sites of atherosclerosis.
3. Develop a lab-on-a-chip technology for detection of activated monocytes based on high affinity CD18 in whole blood.

Introduction/Business Opportunity

Atherosclerosis associated with hyperlipidemia is a complex inflammatory process, characterized pathologically in both humans and mice by recruitment of the monocytic leukocytes in the arterial wall and accumulation of lipid in monocytic leukocytes. Leukocyte recruitment requires adhesion and transendothelial migration, which are mediated by interactions between adhesion molecules. E-selectin, very late antigen 4 (VLA-4, $\alpha 4\beta 1$ integrin), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) have all been shown to play a partial role in monocyte interaction with endothelial cells (ECs), thereby contributing to atherogenesis. Macrophage uptake of modified low density lipoprotein (LDL), resulting in lipid accumulation in macrophages, and foam cell formation, has been considered to be primarily mediated by scavenger receptors (SRs). Initial studies showed that scavenger receptor type A (SR-A), CD36 were centrally implicated in lipid uptake by macrophages, and may play important roles in atherosclerosis. However, the role of the SRs in atherogenesis was not consistently confirmed by other studies. For example, an in vivo animal study showed that circulating monocytes or arterial intimal macrophages may take up lipids independent of the SRs. Therefore, the mechanisms for atherogenesis including those for leukocyte recruitment and lipid accumulation are still not fully understood.

CD11c/CD18, also known as p150,95, $\alpha X\beta 2$, or complement receptor 4, is a member of the leukocyte CD11/CD18 ($\beta 2$) integrins, which also include CD11a, b, and d. In humans, CD11c is found on monocytes/macrophages, granulocytes, activated B cells, and subsets of dendritic cells (DCs). In mice, CD11c is expressed on DCs, and a subset of monocytes/macrophages. In contrast to the well-known functions of CD11a and CD11b in leukocyte adhesion and migration both in vitro and in vivo, CD11c was recently demonstrated to contribute to monocyte capture and transmigration on inflamed ECs by recognizing VCAM-1 (1,2). CD11c also functions as a complement receptor, recognizing iC3b, a cleavage fragment of complement activation product C3b, and participating in the phagocytosis of iC3b-opsonized particles. Deposition of complement activation fragments, C5b-9 and iC3b, has been documented both in human and experimental atherosclerosis. However, the function of iC3b in

atherosclerosis and whether complement receptors including CD11c contribute to the development of atherosclerosis are not clear.

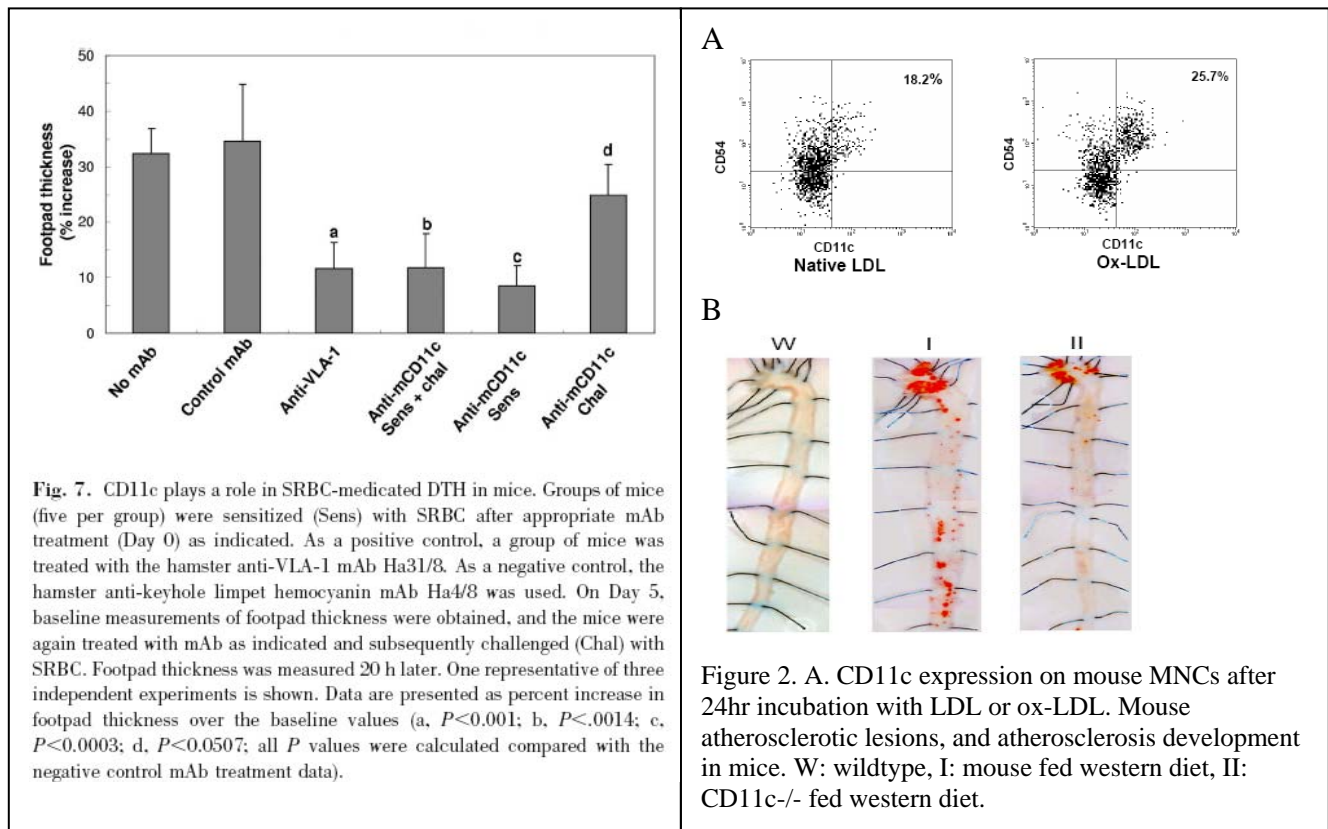
CD11/CD18 integrins have been shown to be involved in atherogenesis as evidenced by a significant reduction in atherosclerosis development in CD18-knockout (KO) mice, which lack all the four CD11/CD18 integrins. In contrast, CD11b expression was not essential for the development of atherosclerosis in LDL receptor-KO mice. Given the functions of CD11c as an adhesion molecules participating in monocyte interaction with ECs, and as a complement receptor mediating phagocytosis of iC3b opsonized particles, we propose that CD11c is a prime target for development of biomarkers for detection and drugs to block the onset of atherosclerosis. If a new small molecule resulting in antagonism of initial trafficking of activated monocytes to athero-susceptible vessels were identified, it would work in tandem with statins and significantly add to the prophylactic strategies in fighting the number one killer of Americans- atherosclerosis.

Core Technology

Dr. Simon at UC Davis, in collaboration with Dr. Ballantyne at Baylor College of Medicine, were the first to identify two key roles of CD11c in atherogenesis. These studies have lead to initial development of antibodies and small molecules that specifically block CD11c function We have further confirmed the validity of CD11c as a target for anti-atherogenic therapies using CD11c/ApoE knockout mouse model that identified three critical functions:

1. CD11c mediated uptake of oxidized lipids by peripheral blood mouse monocytes leading to their activation
2. CD11c binding to atherogenic endothelium recognizes VCAM-1 and is critical for monocyte anchoring and adventitial migration

Our initial strategy is based on studies using antibodies to CD11c to block its binding function in a mouse delayed-type hypersensitivity response model (Figure 1) and in a transgenic mouse model of atherosclerosis (Figure 2).



The data are consistent with the conclusion that CD11c and VLA-4 are equally important in mediating arrest and transmigration of human monocytes on atherogenic HAECs under shear stress (Figure 1). A smaller contribution to adhesion and migration (i.e., ~10%) was detected for CD11a/CD11b binding to ICAM-1. A previous report has demonstrated that CD11a and CD11b interacting with ICAM-1 and ICAM-2 were essential for monocyte transmigration across cytokine-inflamed, venous endothelium in a static adhesion assay. This underscores the prominence of $\alpha 4\beta 1$, CD11c, and VCAM-1 and ICAM-1 interactions under conditions of shear stress, as blocking integrin or VCAM-1 also decreases the efficiency of initial tethering on lipid-primed and cytokine-inflamed aortic endothelium.

The transgenic mouse model underscores the importance of CD11c in the progression of atherosclerosis. Incubation with oxidized lipids leads to monocyte activation, principally, increased CD11c expression (Figure 2A). In a mouse in which hyperlipidemia has been artificially induced, apoE^{-/-} mice fed a high fat diet, removal of CD11c from the mouse decreases the incidence of atherosclerosis (Figure 2B). Taken together the data indicate that during hyperlipidemia, a risk factor for development of cardiovascular disease, CD11c functions in monocyte activation and subsequent recruitment to the vessel wall. We hypothesize that targeting CD11c with blocking agents during early atherosclerosis may decrease the incidences of its clinical manifestations- stroke and myocardial infarction.

Stage of Technology Development

Completed Milestones

1. Demonstrated using an *in vivo* transgenic model of atherosclerosis that CD11c is critical to monocyte uptake of oxidized lipid, recruitment to aorta, and adventitial migration of macrophages in a mouse atherosclerosis model of plaque formation (Submitted *Circulation*).
2. Identified that CD11c is cooperative with VLA-4 in binding VCAM-1 and this is critical to high avidity mediated monocyte adhesion (Sadhu et. al., 2007 *J Leuko Biol*).
3. Validated CD11c as a novel target for antibody treatment of delayed-type hypersensitivity response in mouse model (Sadhu et. al., 2007 *J Leuko Biol*).
4. Initiated studies of CD11c upregulation as a biomarker for incipient monocyte activation in post-prandial whole blood samples in humans.

Proposed milestones

1. Use antibodies to allosteric epitopes on CD11c and CD18 to establish mechanism by which CD11c maintains VLA-4 in high avidity state on activated monocytes. These are owned by Lilly and are denoted:
 - 496K - CD11c allosteric inhibitor antibody
 - 496B - CD11c activating antibody
 - 240Q - CD18 activating antibody
 - 327c - antibody recognizing activated CD18
2. Demonstrate the efficacy of humanized anti-CD11c in blocking monocyte recruitment to atherogenic endothelium in a vascular mimetic lab-on-a-chip assay to predict dose and kinetics targets for further drug development.
3. Perform initial screening of small molecules to CD11c I-domain and establish efficacy of blocking binding to VCAM-1 in recombinant and cell based assay
4. Test small molecule and humanized anti-CD11c in mouse ApoE^{-/-} model of atherosclerosis
5. Establish a lead pro-drug candidate and begin IND application.

Intellectual Property

Need to file a patent disclosure for use of small molecules targeted to conformational shift of CD11c as a novel means of blocking monocyte immune and inflammatory function.

Selected Publications

1. Ting HJ, Stice J, Schaff UY, Hui DY, Rutledge JC, Knowlton AA, Passerini AG, and SI Simon. Triglyceride-rich lipoproteins prime aortic endothelium for an enhanced inflammatory response to TNF- α . 2007. *Circulation Research*. Apr;7(4):448-56.
2. Sadhu C, Ting HJ, Lipsky B, Hensley K, Garcia-Martinez LF, Simon SI, and Donald E. Staunton CD11c/CD18: Novel Ligands and a Role in Delayed Type Hypersensitivity. 2007; *Journal of Leukocyte Biology*. Jun;81(6):1395-403.
3. Huaizhu Wu, Michael Gower, Hong Wang, Xiao-Yuan Dai Perrard, Ruidong Ma, Daniel C. Bullard, Alan R. Burns, Antoni Paul, C. Wayne Smith, MD, Scott I. Simon, and Christie M. Ballantyne, Functional role of CD11c+ monocytes in atherogenesis associated with hypercholesterolemia *Circulation* [submitted]