ARIC Manuscript Proposal # 1218

PC Reviewed: __2/_13/06	**Status: __A__**	**Priority: __2_**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: **Peripheral blood monocyte toll-like receptors TLR-2 and TLR-4 expression and carotid artery atherosclerosis(ARIC CAR MRI Study)**

b. Abbreviated Title (Length 26 characters):
Monocyte TLRs

2. Writing Group:
Writing group members:
Nena Matijevic, Kenneth Wu, Shuyu Yang, Willa Wang, Aaron Folsom, Lloyd Chambless/Diane Catellier (or else from the CC), Brad Astor, Christie Ballantyne, Richey Sharrett.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _ NM____ **[please confirm with your initials electronically or in writing]**

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3. Timeline:
We hope to have a draft manuscript by March 2007.

4. Rationale:
In order to estimate the ability of peripheral blood circulating cellular markers to reflect the inflammatory alterations of the atherosclerotic plaques in carotid atherosclerosis, we will analyze the relation between the whole blood monocyte surface expression of toll-like receptors -2 and -4 and atherosclerotic plaque presence/progression in the carotid artery.
Immune and inflammatory mechanisms are considered to play a key role in the pathogensis of atherosclerosis. Many inflammatory blood and vascular cell types and their activation markers play a role in the initiation, progression and all stages of lesion development. Cell activation and cell-cell interactions result in the production of a cascade of cytokines, chemokines, adhesion molecules, reactive oxygen species and other proinflammatory molecules that contribute to the disease.

The members of the toll-like receptor (TLR) family play a critical role in the inflammatory components of atherosclerosis. Toll-like receptors are expressed preferentially on monocytes/macrophages which play a critical role in recognizing microbial pathogens and produce proinflammatory cytokines. Immunohistochemical studies have demonstrated the presence of TLRs in both human plaques and murine models of atherosclerosis. When TLRs on monocytes/macrophages are activated, they associate with additional membrane proteins and tranduce signals (NFkB pathway) that induce pro-inflammatory cytokines. Among the 10 TLRs identified, TLR-4 is the best characterized. Monocyte CD14 and TLR-4 are two components of the LPS (endotoxin) receptor complex which recognizes gram-negative bacteria and their toxins, has also been reported to be a receptor for endogenous ligands such as fibrinogen, fibronectin, heat-shock protein. TLR-2 responds to various bacterial products including lipoproteins and gram-positive bacterial peptidoglycan. Local arterial TLR-2 stimulation induced intimal hyperplasia and atherosclerotic lesion development in mouse femoral arteries.

Atherosclerosis has been associated not only with local inflammation in the arterial walls but also with the systematic inflammatory response. Since the activation of TLR pathways play an important role in the progression of atherosclerosis, the measurement of the expression levels of TLR2 and TLR4 on circulating monocytes may reflect the inflammatory alterations of the atherosclerotic plaques in carotid atherosclerosis.

In this study, we measured the TLR-2 and TLR-4 levels in the peripheral blood monocytes of the ARIC CAR MRI study participants by flow cytometry. Whole blood monocytes were labeled with monoclonal antibody to the surface receptor CD14 and monoclonal antibodies to the surface receptors TLR-2 and TLR-4.

5. Main Hypothesis/Study Questions:

a) Peripheral blood monocytes TLR-2 and TLR-4 reflect the inflammatory alterations of the atherosclerotic plaques.
b) Patients with increased wall volume have increased levels of the TLR-2 and TLR-4 on circulating monocytes.
c) Monocyte TLR-2 and -4 levels are independently associated with carotid artery plaque presence/progression in atherosclerotic patients.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Exclusions: missing covariates, any cell marker that proved not to be reliable.

Independent variables: CD14 (MFI), TLR-2 (percentage of CD14 positive monocytes expressing TLR-2 and the level of its expression (MFI)), TLR-4 (percentage of CD14 positive monocytes expressing TLR-4 and the level of its expression (MFI), CD41 (%), CD162 (MFI). We will also analyze the relation between the level of CD14 (MFI) expressed by TLR (+) vs TLR(-) monocytes, and level of PSGL-1 (CD162; MFI) expressed by TLR-2 (+) vs TLR-2(-) monocytes, We will analyze the relation between the TLR-4 levels in CD14(+) CD41 (+) vs CD14(+) CD41(-) monocytes in order to see if binding of platelets (CD41) to monocytes (CD14) will alter the TLR-4 expression levels.

The association of the independent variables with the MRI dependent variables (wall volume, lipid core volume, fibrous cap thickness, etc) will be assessed by linear or logistic regression (the latter for any categorical MRI variables), adjusting for age, race, sex, and additional by other covariates listed below.

Covariates: basic risk factors (age, race, gender, LDL-C, HDL-C, lipid med use, systolic BP, antihypertensive med use, diabetes, obesity, cigarette smoking status, alcohol intake, physical activity, BMI, waist to hip ratio, and CRP). All will be from the MRI visit.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes _xx___ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No

(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes __xx__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"?
____ Yes ____ No

9.The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

__xx____ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __xx__ No

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study (list number* _____)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.