Brachial Artery Vasodilator Function and Systemic Inflammation in the Framingham Offspring Study

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Background—In experimental studies, traditional risk factors and proinflammatory processes alter the regulatory functions of the vascular endothelium to promote atherosclerosis. These alterations include expression of leukocyte adhesion molecules and decreased bioavailability of endothelium-derived nitric oxide, an important regulator of vascular homeostasis and tone. The precise relations among risk factors, inflammation, and nitric oxide bioavailability remain uncertain.

Methods and Results—To test the hypothesis that inflammation impairs endothelial function in humans, we measured brachial artery flow-mediated dilation, reactive hyperemia, and serum concentrations of C-reactive protein (CRP), interleukin-6 (IL-6), soluble intracellular adhesion molecule-1 (sICAM-1), and monocyte chemotactic protein-1 (MCP-1) in 2701 participants from the Framingham Study (mean age 61 years, 53% women). There were modest unadjusted inverse correlations between flow-mediated dilation and CRP, IL-6, and sICAM-1 ($P<0.001$ for all) that were rendered nonsignificant after accounting for traditional coronary risk factors. For reactive hyperemia, we observed inverse correlations with markers of inflammation in unadjusted models that were attenuated 57% to 74% after accounting for risk factors. However, partial correlations of CRP, IL-6, and sICAM-1 with reactive hyperemia remained significant.

Conclusions—Our observations are consistent with the hypothesis that risk factors induce a state of inflammation that impairs vascular function. For flow-mediated dilation, we found no evidence that inflammation has additional effects beyond those attributable to traditional risk factors. The incremental contribution of CRP, IL-6, and sICAM-1 to reactive hyperemia above and beyond known risk factors suggests that systemic inflammation may contribute to impaired vasomotor function in forearm microvessels. (Circulation. 2004;110:3604-3609.)

Key Words: endothelium $\blacktriangleright$ inflammation $\blacktriangleright$ atherosclerosis $\blacktriangleright$ risk factors $\blacktriangleright$ cardiovascular diseases

The inflammatory nature of atherosclerosis is now well accepted.1–3 During atherogenesis, recruitment of leukocytes to the arterial wall promotes the development of atherosclerotic lesions. Later in the disease, inflammation promotes plaque rupture and acute coronary syndromes. In support of these concepts, serum markers of inflammation, including C-reactive protein (CRP), interleukin-6 (IL-6), the soluble form of intercellular adhesion molecule-1 (sICAM-1), and monocyte chemotactic protein-1 (MCP-1), predict cardiovascular disease events.3

The vascular endothelium contributes to inflammatory responses in atherosclerosis.1,2,4 Experimental studies indicate that certain cardiovascular risk factors and proinflammatory cytokines activate endothelial cells to express leukocyte adhesion molecules, which promotes adhesion of monocytes and T lymphocytes to the endothelial surface. Endothelial cells and other vascular cells also produce MCP-1 and other chemoattractants that promote leukocyte accumulation in the vascular wall. The presence of cardiovascular disease risk factors, cytokines, and inflammatory factors such as CRP induces important phenotypic changes in the endothelium, including a decrease in the production and/or biological activity of endothelium-derived nitric oxide.3,5 Decreased nitric oxide activity promotes leukocyte adhesion, thrombosis, vasoconstriction, and cellular proliferation and thus plays a role in all stages of atherosclerosis.6

Previous human studies examining the relation between endothelial function and specific markers of inflammation...
have yielded inconsistent results, although much of that work involved relatively small numbers of highly selected individuals. The present study examined the cross-sectional relations between vasodilator function in the forearm (brachial artery flow-mediated dilation and reactive hyperemia) and 4 systemic markers of inflammation (CRP, IL-6, sICAM-1, and MCP-1), while adjusting for known risk factors in a large, community-based sample.

Methods

Participants

The design for the Framingham Offspring Study has been described elsewhere. Participants in the seventh examination cycle (1998 to 2001) were eligible for the present investigation (n=3539). Participants were excluded for the following reasons: nursing home examination (n=205), missing covariate data (n=12), missing inflammatory marker data (n=170), and the unavailability of 2D ultrasound data as described previously, which left a total of 2701 individuals for investigation of flow-mediated dilation. For investigation of the relation between reactive hyperemia and markers of inflammation, we limited the analysis to the 1903 participants with Doppler flow data. All participants underwent routine medical history, physical examination, and laboratory assessment. Individuals were instructed not to eat or drink anything except water or decaffeinated black coffee or tea after 8 PM the previous evening. Cigarette smoking was determined by self-report. Heart rate and blood pressure were measured by an automatic device (Dinamap, model No. 1846SX, Critikon, Inc). Diabetes was defined as fasting glucose \( \geq 126 \text{ mg/dL} \) or use of hypoglycemic medication. Prior cardiovascular disease (coronary heart disease, stroke, intermittent claudication, or congestive heart failure) was documented as described previously. The examination included a 6-minute exercise test (Bruce protocol stages I and II) in participants without contraindications (known coronary heart disease, chest pain on test day, or inability to perform test). The Boston Medical Center Institutional Review Board approved the study, and all participants provided written informed consent.

Determination of Flow-Mediated Dilation and Reactive Hyperemia

The method and reproducibility for determining flow-mediated dilation have been described previously. Briefly, a Toshiba SSH-140A ultrasound system with 7.5-MHz linear array transducer (Toshiba Medical) was used to record 2D images of the brachial artery at baseline and 1 minute after induction of reactive hyperemia by 5-minute cuff occlusion of the forearm. Using a carrier frequency of 3.75 MHz and correction for insonation angle, Doppler flow was assessed at baseline and during peak hyperemic flow, which was visually confirmed to occur within 15 seconds after cuff release. The Doppler flow signals and 2D images were captured digitally with customized equipment (Cardiovascular Engineering, Inc).

Participants examined the correlations between each marker of inflammation with each measure of flow-mediated dilation and flow. We estimated crude correlations, partial correlations that accounted for gender and age, and partial correlations that accounted for gender, age, and multiple clinical variables using SAS CORR procedure. A 2-sided probability value <0.05 was considered statistically significant.

Results

Participant Characteristics and Markers of Inflammation

The clinical characteristics of the participants are displayed in Table 1 and are very similar to the characteristics of other subsets of Framingham Offspring participants used in prior studies. Summary statistics for the measures of vascular function and inflammation are provided in Table 2. Unadjusted correlates among the 4 markers of inflammation were shown in Table 3 and reveal that the inflammatory markers were correlated, particularly CRP and IL-6, which had an unadjusted correlation coefficient of nearly 0.5.

Markers of Inflammation and Flow-Mediated Dilation

To investigate the hypothesis that systemic inflammation adversely affects conduit artery endothelial function, we examined the correlations between each marker of inflammation and brachial artery flow-mediated dilation (Table 4).
observed significant inverse correlations between flow-mediated dilation and CRP, IL-6, and sICAM-1 and a correlation of borderline significance with MCP-1. After adjustment for traditional risk factors, the correlations were no longer statistically significant for CRP (Figure), IL-6, sICAM-1, and MCP-1. The multivariable model contained traditional cardiovascular disease risk factors and the other significant multivariable predictors of flow-mediated dilation identified in a previous study and included age, gender, body mass index, systolic blood pressure, total cholesterol:HDL cholesterol ratio, diabetes mellitus, recent cigarette smoking, hormone replacement therapy, lipid-lowering therapy, heart rate, and whether or not the walk test was performed before the study of vascular function. The findings were similar whether flow-mediated dilation was expressed as absolute change or as percent change in diameter.

Markers of Inflammation and Brachial Flow

Because hyperemic flow is the principal stimulus for flow-mediated dilation and an index of microvascular function, we investigated the correlations between each marker of inflammation and the extent of reactive hyperemia (Table 4). Reactive hyperemia correlated inversely with CRP, IL-6, sICAM-1, and MCP-1 in the unadjusted models. After adjustment for risk factors, the correlations were no longer statistically significant for MCP-1. For CRP, IL-6, and sICAM-1, the correlation coefficients were reduced by 57% to 74% but remained statistically significant in the multivariable models (Figure). The findings were similar whether the extent of reactive hyperemia was expressed as hyperemic flow or as the ratio of hyperemic flow to baseline flow, as it has often been expressed in prior studies. Given that baseline flow may also be a determinant of endothelial expression of inflammatory factors, we examined the relation between the markers of inflammation and baseline flow. We found modest inverse correlations with CRP, IL-6, and sICAM-1 but no relation with MCP-1 in the unadjusted models. After adjustment for age, gender, and other risk factors, we found no significant correlation between baseline flow and any of the markers of inflammation.

Possible Effects of Cardiovascular Disease and Cardiovascular Medications

Some of the participants had cardiovascular disease or were taking hormone replacement or lipid-lowering therapy (Table 1), and we considered the possibility that these factors might have confounded our results. We therefore repeated the analyses of conduit brachial artery flow-mediated dilation and extent of reactive hyperemia in the subgroups of participants without known cardiovascular disease, not receiving hormone replacement therapy, and not receiving lipid-lowering therapy. In each subgroup, the results were not materially different from those for the entire cohort (data not shown).

Discussion

In our community-based sample, we investigated the relations of markers of inflammation to flow-mediated dilation, a measure of conduit artery vasodilator function, and to reactive hyperemia, a measure of forearm microvascular vasodilator function. We observed modest unadjusted correlations between flow-mediated dilation and the inflammatory markers; however, these relations were rendered nonsignificant on adjustment for cardiovascular disease risk factors. We observed a similar pattern of statistically significant unadjusted correlations and weakened risk factor–adjusted correlations between all 4 markers of inflammation and reactive hyperemia. CRP, IL-6, and sICAM-1 remained significant correlates of microvascular function after adjustment for cardiovascular risk factors.

Investigators have hypothesized that inflammation is an important cause of endothelial dysfunction, but prior human studies relating inflammation to endothelium-dependent dilation have been limited to relatively small and selected samples and yielded conflicting results. For example, Fichtlscherer and colleagues reported that CRP correlated with endothelial dysfunction in forearm microvessels in coronary disease patients and that time-dependent improvements in endothelial function paralleled reductions in CRP. Several groups also reported a significant correlation between CRP and lower brachial artery flow-mediated dilation. In the coronary circulation, CRP levels correlated with more severe constrictor responses to the cold pressor test, which may reflect, in part, endothelial dysfunction. In contrast, CRP did not correlate with coronary endothelial dysfunction (assessed by acetylcholine infusion) in patients with coronary
artery disease and risk factors. Moreover, CRP did not correlate with brachial artery flow-mediated dilation in patients with familial hypercholesterolemia. Conflicting results have also been reported with regard to the relation between inflammatory markers and microvascular function. This finding raises the possibility that systemic inflammation may represent a mechanistic link between risk factors and vascular dysfunction. Flow-mediated dilation represents an important homeostatic response to short-term changes in shear stress at the endothelial surface and depends on production of nitric oxide, particularly in conduit arteries. Our findings for reactive hyperemia may be of particular interest, however, because adjustment for risk factors did not completely abrogate the relation between inflammatory markers and microvascular function. This finding raises the possibility that some component of inflammation or a source of inflammation in addition to traditional risk factors might contribute to microvascular dysfunction. Overall, these findings support the possibility that systemic inflammation may represent a mechanistic link between risk factors and vascular dysfunction.

The present study may provide a basis for reconciling this conflicting literature. The previous studies had limited statistical power to adjust for risk factors, whereas the present study had a sample size 10-fold larger than any prior report. We found that adjustment for standard cardiovascular disease risk factors markedly weakened the relation between inflammation and vascular function, which suggests that these risk factors may be the primary driving force for vascular dysfunction. Thus, the possibility that systemic inflammation may represent a mechanistic link between risk factors and vascular dysfunction.

Unadjusted and partial correlations between CRP and vascular function. Displayed are unadjusted and partial Spearman correlations for CRP with flow-mediated dilation (FMD; left) and CRP with reactive hyperemia (right). As shown, correlations were markedly attenuated when we accounted for age and gender and for individual and multiple risk factors as described in Results. DM indicates diabetes mellitus; BMI, body mass index.
prostacyclin, and other vasodilators by the endothelium.\textsuperscript{17} Inflammation has the potential to impair flow-mediated dilation by reducing the bioavailability of endothelium-derived vasodilators. For example, experimental studies have shown that CRP decreases expression of endothelial nitric oxide synthase (eNOS) and nitric oxide synthesis, in part by decreasing the half-life of eNOS message.\textsuperscript{5} These effects of CRP might also account for the relation between IL-6 and flow-mediated dilation, because IL-6 is an important stimulus for CRP production in the liver.\textsuperscript{3} The strong correlation between CRP and IL-6 observed in the present study is consistent with this possibility (Table 3); however, we cannot exclude the possibility that CRP, IL-6, and sICAM-1 are merely markers for the presence of other factors that account mechanistically for the observed variation in vascular function.

Reactive hyperemia is a complex response that reflects dilation of microvessels by non–endothelium-dependent vasodilators generated during local ischemia, including adenosine.\textsuperscript{17} In addition, there is growing recognition that endothelium-derived nitric oxide contributes to reactive hyperemia, as evidenced by reductions in both the peak and overall flow response (area under the curve) during concomitant infusion of eNOS inhibitors.\textsuperscript{26–28} Risk factors and atherosclerosis are associated with a reduced peak hyperemic response,\textsuperscript{13} particularly with a loss of the nitric oxide–dependent portion of the response.\textsuperscript{26,29} Thus, the observed relation between markers of inflammation and lower reactive hyperemia might reflect endothelial dysfunction in the microvasculature. Alternatively, the findings could be attributable to other aspects of vascular dysfunction, including reduced production of non–endothelium-dependent dilators, altered function of vascular smooth muscle, or structural changes in the microvasculature that limit dilation.\textsuperscript{30,31} Further studies would be needed to investigate these possibilities.

Reactive hyperemia and flow-mediated dilation are interrelated. Reactive hyperemia is the stimulus for flow-mediated dilation of the conduit artery. Conversely, flow-mediated dilation of microvessels augments the hyperemic response. The correlation coefficients relating inflammation to hyperemic flow were 2 to 3 times higher than the corresponding correlation coefficients for flow-mediated dilation. We recently observed an inverse correlation between hyperemic flow and traditional risk factors, which provides evidence that risk factors may impair flow-mediated dilation in part by reducing the stimulus for dilation.\textsuperscript{13} It is possible that inflammation has a predominant effect on the microvasculature rather than the conduit artery in the forearm. The extent to which this impairment of microvascular function reflects “endothelial dysfunction” rather than “vascular dysfunction” remains unknown.

We observed only very modest unadjusted correlations between MCP-1 and flow-mediated dilation, and the relations with reactive hyperemia did not persist after adjustment for risk factors. Experimental studies suggest an inhibitory effect of nitric oxide on MCP-1 expression,\textsuperscript{32} and many of the same stimuli that promote MCP-1 expression by vascular cells are also associated with decreased bioavailability of nitric oxide.\textsuperscript{13} Thus, it is unclear why a stronger relation was not observed. It is possible that serum levels of MCP-1 do not reflect the local vascular wall milieu to the same extent that the soluble form of ICAM-1 reflects expression at the luminal surface of the endothelium.

The present study has several limitations. We emphasize that this cross-sectional study cannot establish mechanistic links between risk factors, systemic inflammation, and vascular function, although our results are consistent with this possibility. The findings could be explained by novel risk factors or genetic polymorphisms that were not included in our models. It also remains possible, although unlikely, that inflammation and vascular dysfunction are entirely distinct phenomena produced separately by risk factors. In addition, our cohort was predominantly white, and our finding may not apply to other ethnic/racial groups. Finally, flow-mediated dilation was relatively low compared with some prior studies, which likely reflects the older age of the participants and the below-elbow cuff position,\textsuperscript{44} although results of the present study are similar to those of prior studies that used the same cuff position and comparable subjects.\textsuperscript{35,36} It remains possible that a very modest correlation between flow-mediated dilation and markers of inflammation was undetected. In spite of these limitations, the present study has several strengths, which include a community-based cohort, which enhances generalizability, and the large sample size, which provides excellent power and the ability to adjust for multiple routinely ascertained covariates.

In conclusion, traditional cardiovascular disease risk factors appear to account for much of the relation between serum markers of inflammation and 2 aspects of vascular function, specifically brachial artery flow-mediated dilation and reactive hyperemia, in this community-based cohort. These observations are consistent with the hypothesis that traditional risk factors induce a state of inflammation that impairs endothelial and/or vascular function in the forearm. Our findings may have clinical relevance, because endothelial dysfunction is strongly linked to the pathogenesis and clinical expression of atherosclerosis.\textsuperscript{16} Recent studies suggest that traditional risk factors are the primary driving force for cardiovascular risk,\textsuperscript{37,38} and the present study may be consistent with those observations. Longitudinal studies in this and other cohorts may provide additional information about the links between systemic inflammation, vascular dysfunction, and cardiovascular disease.

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