Letters to the Editor

Genetic Association Studies

To the Editor:
I am delighted to see that Arteriosclerosis, Thrombosis, and Vascular Biology has taken the lead in initiating an open discussion about genetic association studies in such a prominent manner. As correctly pointed out in the editorial by Dr Hegele,1 the number of publications has exponentially increased in the last decade. Despite the intrinsic problems with this approach, this exponential growth also demonstrates the popularity of this type of study. Anyone who follows the literature in genetic association studies will recognize, as Dr Hegele does, that none of the polymorphisms reported so far has been consistently associated with any phenotypic trait, either a single quantitative trait such as triglyceride level or a complex trait such as atherosclerosis. A typical example is the polymorphisms in endothelial NO synthase,2 which has been associated with various vascular diseases in some studies but not in others. Among many reasons for such inconsistent association studies, the importance of controlling for environmental influence should be specifically emphasized because it can modify the phenotypic effect of a given genotype and potentially alter the direction of the association.3,4 However, we should also not deny the fact that these association studies have stimulated many mechanistic investigations, often from a biochemically unconventional angle, which have the potential for novel findings. While I agree with most criteria set out by Dr Hegele and Drs Almasy and MacCluer, particularly multi-focus approaches and haplotype analyses, one must be aware that not many research groups have the resources to satisfy them all. This is particularly true in some countries with small research budgets, where investigators often have access to patients with unique phenotypes and ethnic backgrounds. Denying public access to these unique data, although they may be statistically powerless, would minimize our chances for some novel discoveries. I applaud the suggestion of a shorter or electronic format for publication and support the criteria set out by Dr Hegele. I suggest that we should take one step further in a spirit similar to that of GenBank. Authors could be asked, when they submit a manuscript to ATVB, that they also deposit the original data in Excel (Microsoft) format to a specific ATVB database with some standardized entries such as age, sex, ethnicity. This database could be established under a heading for each specific phenotype, such as hypercholesterolemia. When the database has accumulated to a level that statistical power will be adequate, ATVB could commission meta-analyses. This would not only generate some definitive data that would satisfy criteria set down by statisticians, but it would also acknowledge the contributions of individual investigators to the genetic associations.

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Periprocedural Kinetics in Serum Levels of Cytokines and Adhesion Molecules in Elective PTCA and Stent Implantation: Impact on Restenosis

To the Editor:
Coronary restenosis after percutaneous transluminal coronary angioplasty (PTCA) remains a significant clinical problem occurring in ~30% of patients even after wide accessibility and application of intraluminal stent implantation. Recent developments in stent design have made new techniques such as brachytherapy and drug-eluting stents available for clinical approaches; nonetheless, restenosis with its complex multifactorial genesis will continue to be an important problem with a major impact on long-term outcome of patients after coronary interventions. Several factors have been suggested to serve as predictors of the later occurrence of restenosis, but no serum parameter with predictive value has been clinically established so far.

The pathophysiology of coronary restenosis has not been fully elucidated; however, an initial vascular injury is supposed to initiate a local inflammatory response that induces the recruitment of circulating immune-competent cells through an increased expression and translocation of several adhesion molecules such as P-selectin, E-selectin, and monocyte chemoattractant molecule-1 (MCP-1). Migration of leukocytes, release of cytokines, and growth factors result in the induction of local inflammatory reactions and vascular remodeling. Patients with angina pectoris and acute myocardial infarction show a systemic inflammatory activation and elevated serum levels of vascular adhesion molecules. Therefore, the local vascular inflammation seems to be accompanied by alterations of serum parameters exhibiting a systemic inflammatory response toward local tissue damage.

We investigated periprocedural serum kinetics of cytokines (TNFα and IL-1β) and adhesion molecules (ICAM-1, VCAM-1, E-selectin and P-selectin) from baseline to 48 hours after elective PTCA and intracoronary stent implantation in patients with stable angina pectoris (CCS II-III). Forty patients with stable angina pectoris and a left ventricular ejection fraction of >45% admitted for elective PTCA and stent implantation were prospectively included in this study. Before and 48 hours after intervention, venous blood samples were drawn from all individuals to assess kinetics in serum parameters over the periprocedural period. Control coronary angiography was performed after 6 months to assess the degree of in-stent restenosis. Angiograms were analyzed for degree of stenosis before and after stent implantation and at follow-up angiograms by using Quantitative Coronary Analysis (MEDIS). Restenosis was defined as >50% recurrent lumen diameter stenosis.

All patients received aspirin (100 mg daily) for the entire study period. For 4 weeks after stent implantation, all patients were administered an initial loading dose of 300 mg clopidogrel followed by 75 mg of clopidogrel daily. Furthermore, all patients received a standard medication of statins, β-blockers, and an ACE-inhibitor.

Of the 40 patients initially included in this study, control angiography could be performed in 36 patients. Eleven (31%) patients showed evidence of restenosis (>50% diameter stenosis). Twenty-seven (75%) patients had one or more cardiovascular risk factors (hypertension, hyperlipidemia, diabetes mellitus, smoking). Twenty-five (69%) patients were on antihypertensive treatment. Sixteen (44%) received lipid lowering medication. Diabetes mellitus was present in 9 (25%) patients. Fifteen (42%) patients were present or previous smokers (P was not significant for risk factors, baseline medication, and gender). One-vessel disease was present in 16 (47%) patients, two-vessel disease in 12 (33%) patients, and three-vessel disease in eight (22%) patients. Direct stent implantation was performed in 16 (44%) patients, whereas in the other 20 (56%) patients, the stent was implanted after predilatation with a conventional balloon. In 83% of the patients, a single stent was implanted. Four (13%) patients received two stents and one (4%) patient three stents.

All patients undergoing PTCA and stent placement showed a significant increase in serum TNFα (baseline, 1.16±0.20 pg/mL; 48 hours, 1.47±0.26 pg/mL; P<0.05; Figure). Serum levels of IL-1β did not change significantly in the whole study population. Patients with restenosis showed a slightly more pronounced but not significant increase in TNFα (P=0.64). Serum levels of IL-1β increased only in patients who developed restenosis (baseline, 0.17±0.13 pg/mL; 48 hours, 0.59±0.29 pg/mL; P<0.0001). In contrast, patients without restenosis showed a decrease in IL-1β (baseline,
Changes in serum concentration of proinflammatory cytokines (TNFα and IL-1β) and soluble adhesion molecules (ICAM-1 and VCAM-1) within 48 hours after PTCA and stent implantation. The serum concentration of IL-1β increased in the peri-procedural period in patients who developed intracoronary restenosis whereas patients without restenosis exhibited a decrease in serum levels of IL-1β. Changes in serum levels of TNFα showed no significant differences between the two groups. In patients who remained free of restenosis, serum levels of ICAM-1 and VCAM-1 showed a significant decrease within 48 hours after intracoronary intervention but did not change significantly in patients who developed in-stent restenosis.

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5. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tietz L, Meyer J, AtheroGene Investigators. Circulating cell adhesion molecules and adhering platelets. After injury, intercellular adhesion molecules induce the recruitment and migration of mononuclear cells. Even though the origins of circulating adhesion molecules are not entirely clear, a main part seems to result from shedding or proteolytic cleavage from the cell reflecting the expression of membrane-bound adhesion molecules. In patients developing restenosis after PTCA, levels of MCP-1 increased chronically with predictive value in regard to late lumen loss. Increased serum levels of soluble adhesion molecules have also been reported in patients with unstable angina and following intravascular intervention with an impact on morbidity and mortality.

In conclusion, our findings suggest that patients at increased risk of restenosis following intracoronary intervention can be detected by a distinct time course of inflammatory serum parameters in the peri-procedural period and, therefore, may profit from an early initiation of additional anti-inflammatory therapies. An advantage of our approach is the clinical practicability through a relatively easy routine measurement of serum parameters immediately before and 48 hours after intervention. The present study supports the hypothesis that inflammatory mechanisms in response to vascular injury are at least in part responsible for intracoronary restenosis and abnormalities can be detected in the peripheral blood stream.

**Letters to the Editor**

**0.19 pg/mL vs 0.18 ng/mL;**

**0.12 ng/mL;**

**0.050 pg/mL;**

**0.078 ng/mL; 48 hours, 0.27 pg/mL;**

**0.24 pg/mL;**

**0.21 ng/mL;**

**0.20 pg/mL;**

**0.25 ng/mL;**

**0.23 pg/mL; 48 hours, 0.27 pg/mL;**

**0.05 versus baseline; #P<0.05 versus no restenosis.**

*These authors contributed equally to this work.*

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In Response:

Shaskin et al show that SR-PSOX (CXCL16) mRNA is expressed in human T cells from peripheral blood using quantitative real-time RT-PCR. In accordance with our findings on SR-PSOX mRNA expression in smooth muscle and endothelial cells, their results indicate that expression of this scavenger receptor is not exclusively a feature of antigen-presenting cells. However, they report that SR-PSOX mRNA expression in T cells and human monocyte-derived macrophages (MΦ) is strongly downregulated following phorbol 12-myristate 13-acetate (PMA)/ionomycin induction, whereas Shimaoka et al found upregulation of SR-PSOX mRNA and protein in THP-1 cells after 3 d of stimulation with 160 nmol/L PMA. In agreement with the results of Shimaoka et al our experiments revealed a 1.7-fold increase in SR-PSOX mRNA expression in THP-1 cells after 1 d incubation with 100 nmol/L PMA, and a 3.8-fold increase after 3 d incubation using RT-PCR (unpublished data). It remains unclear whether the disparate results in these similar experiments are due to the use of PMA/ionomycin by Shaskin et al instead of PMA alone. Further experiments are in progress to elucidate the regulation of SR-PSOX in MΦ and other cell types besides antigen-presenting cells.

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In Response:

By RT-PCR, we also have found that in the human T cell line, Jurkat cells express CXCL16/SR-PSOX mRNA, although their expression levels were much less than those found in THP-1 macrophages (Figure). This seems to be consistent with the observation shown in this letter by Shashkin et al that the CXCL16 mRNA expression level in T lymphocytes was approximately 10% of that in macrophages. Levels of CXCL16/SR-PSOX expression in U937 cells and MM6 cells might be less than those in macrophages. In addition, it remains unclear how much protein is expressed on the cell surface or how much of the protein is released as soluble molecules in T lymphocytes, because RT-PCR alone so far is the evidence for CXCL16/SR-PSOX expression in T cells. Therefore, pathophysiological roles of CXCL16/SR-PSOX in T cells remain totally speculative. Further studies by use of specific antibodies for CXCL16/SR-PSOX, as well as gene knockout mice, would tell us more concerning the roles of CXCL16/SR-PSOX in T cells.

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RT-PCR analysis for CXCL16/SR-PSOX mRNA in various cultured cells. Equal amounts of total cellular RNA (250 ng) was reverse-transcribed with oligo (dT) primer by using SuperScript II Reverse Transcriptase (Invitrogen). Transcribed cDNAs were subjected to PCR with specific primers for human SR-PSOX (5′-ACTCAGCCAGGCAATGGCAAC-3′ and 5′-GGTATTAGATCAGGTCAC-3′), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5′-CGGTCACCACGGCTGTTT-3′ and 5′-CATGAGGGCACCACCCCTTGTT-3′) with Taq DNA polymerase (QIAGEN). PCR products were then subjected to electrophoresis through 1% agarose gels and visualized by ethidium bromide staining. Lane 1, CHO-K1 cells; lane 2, CHO-K1 cells transfected with human SR-PSOX cDNA; lane 3, Jurkat cells; lane 4, THP-1-derived macrophages.
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