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 even change the direction of the association.3,4 However, we should also not deny the fact that these association studies have accumulated to a level that statistical power will be adequate, and the database could be established under a heading for each specific phenotype, such as hypercholesterolemia. When the database has shown evidence of restenosis (50% diameter stenosis). Twenty-five (69%) patients were present or absent in one-vessel disease; present or absent in 16 (44%) patients, whereas in the other 20 (56%) 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 predilation 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 periprocedural 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. (*P<0.0001 versus baseline; †P<0.05 versus baseline; ‡P<0.01 versus no restenosis. □ indicates no restenosis; ■ restenosis.)

0.50±0.24 pg/mL; 48 hours, 0.27±0.27 pg/mL; P<0.05. The difference in changes of IL-1β serum levels was statistically significant between the two subgroups (0.42±0.19 pg/mL vs -0.23±0.20 pg/mL; P<0.01) and showed a significant positive predictive value regarding the later occurrence of restenosis following PTCA and stent implantation (RR: 2.6; CI: 1.3 to 5.4).

The whole study population showed no significant differences in serum levels of ICAM-1, VCAM-1 and P-selectin 48 hours after stent implantation. Only E-selectin exhibited a significant decrease in all patients (2.47±0.22 vs 2.04±0.18 ng/mL; P<0.05). In patients without restenosis, serum levels of ICAM-1 (16.3±1.69 vs 14.7±2.42 ng/mL; P<0.0001) and VCAM-1 (7.86±0.58 vs 7.39±0.63 ng/mL; P<0.0001) showed a significant reduction. In patients with restenosis, ICAM-1 and VCAM-1 serum concentrations remained unchanged. Serum levels of E-selectin decreased in both groups of patients (2.35±0.46 vs 1.91±0.33 ng/mL; P<0.0001; 2.53±0.24 vs 2.1±0.21 ng/mL; P<0.0001). P-selectin decreased only in patients without restenosis (5.03±0.50 vs 4.11±0.50 ng/mL; P<0.001).

The present study demonstrates for the first time periprocedural kinetics in serum levels of cytokines and adhesion molecules within 48 hours after PTCA and intracoronary stent implantation. Our data confirm previous findings and support the hypothesis of a functional modulation of the progression of coronary artery disease by circulating molecules following vascular injury.

Inflammatory processes have been extensively investigated in coronary artery disease.1-5 Circulating immune cells as well as the vascular wall have been shown to contribute to increased systemic levels of cytokines in atherosclerosis and following vascular injury.5 Recently, the cytokine-generating capacities of monocytes (in particular IL-1β) were found increased in patients with restenosis after PTCA.3 Moreover, in experimental carotid artery balloon angioplasty, the local expression of IL-1β and its receptor increased after 6 hours and normalized within 24 hours. IL-1β accumulates with neointimal smooth muscle cells, suggesting a distinct role in neointima formation.7 These studies are well in line with the findings of the present study demonstrating increased systemic levels of proinflammatory cytokines after PTCA and stent implantation in patients developing intracoronary restenosis. The crucial role of IL-1β is further indicated by a pronounced decrease in patients who remain free of restenosis. Therefore, in addition to their role in the local response to vascular injury, proinflammatory cytokines are involved in the systemic inflammatory activation with predictive value in patients after PTCA and stent implantation. One might speculate the periprocedural time course of cytokines and adhesion molecules reflects the degree of vascular injury during the intervention and is also influenced by the specific practice of PTCA and stent implantation.

The decrease in soluble adhesion molecules only in patients without restenosis argues for an involvement of these molecules in the pathogenesis of coronary restenosis. Cellular adhesion molecules are expressed in developing neointima as well as endothelial cells and adhering platelets.2 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 endothelial cells 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.1 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.6-9

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 periprocedural 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.

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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 mmol/L PMA. In agreement with the results of Shimaoka et al our experiments revealed a 1.7fold increase in SR-PSOX mRNA expression in THP-1 cells after 1 d incubation with 100 mmol/L PMA, and a 3.8fold 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 Shaskin 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′-ACTCAGCCAGGCAATGGAAC-3′ and 5′-GTTATAGAGTCAGGTGCCAC-3′), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5′-CTGTCACCAGGGCTGTTT-3′ and 5′-CATGAAGTCAACCACCTGT-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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