Increased Expression of Monocyte CD11a and Intracellular Adhesion Molecule-1 in Patients With Initial Atherosclerotic Coronary Stenosis

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Background Cell adhesion molecules have been implicated in the adhesion of leukocytes to endothelial cells and therefore play a role in atherosclerosis, which is a frequent cause of morbidity and mortality in patients with coronary artery disease (CAD) or undergoing hemodialysis (HD). The levels of expression of leukocyte adhesion molecules were evaluated in patients with CAD or HD.

Methods and Results The expression of leukocyte (ie, neutrophil, monocyte and lymphocyte) surface CD11a, CD18, intracellular adhesion molecule-1 (ICAM-1), very late antigen-4 (VLA-4) and L-selectin was investigated by flow cytometry in 20 patients who were initially diagnosed with CAD (CAD group), 15 patients with coronary re-stenosed vessels (RESTE group), 20 undergoing HD (HD group) and 20 without CAD (CONT group). Monocyte surface expression of both CD11a and ICAM-1 in the CAD group was significantly higher than in the CONT group. Interestingly, when 15 patients with RESTE were analyzed, they showed monocyte CD11a and ICAM-1 expression levels comparable to those in the CONT group. On the other hand, there were no significant differences in the expression of CD11a, CD18, L-selectin or VLA-4 between the HD group and CONT group, but monocyte L-selectin was increased in the CAD group compared with the CONT group.

Conclusions Because CD11a and CD18 are expressed on the cell surface as a heterodimer and ICAM-1 is a ligand for CD11a/CD18, this increased expression of CD11a and ICAM-1 may affect the development of initial atherosclerotic coronary stenosis, but not re-stenosis. (Circ J 2004; 68: 6–10)

Key Words: CD11a/CD18; Coronary artery disease; Hemodialysis; Intracellular adhesion molecule-1

M any recent experimental and clinical studies have suggested that leukocyte adhesion to vascular endothelial cells is an important step in atherosclerosis.1–4 Adhesion molecules are specific proteins on the endothelial cell surface and counter the receptor proteins on the leukocyte surface that regulate the different steps in leukocyte migration from the blood stream into the vessel wall.5–7 The serum concentration of adhesion molecules has been shown to be related to the development of coronary artery disease (CAD)8 including myocardial infarction (MI)9 and post-angioplasty restenosis.10

This adhesion is mediated by adhesion molecules such as CD11a, CD18, intracellular adhesion molecule-1 (ICAM-1), very late antigen-4 (VLA-4) and L-selectin.11 CD11a is a β2-integrin that promotes the adherence of neutrophils and monocytes to ICAM-1 on endothelial cells, and subsequently helps to regulate the adhesive cascade.12,13 CD11a and CD18 are expressed on the cell surface as a heterodimer. ICAM-1 is a ligand for CD11a/CD18 and a member of the immunoglobulin family.14 It is moderately expressed on endothelial cells, lymphocytes and monocytes.12 In addition, the focal expression of ICAM-1 has been demonstrated in human atherosclerotic plaques.15,16 Interestingly, soluble ICAM-1 concentration has been shown to be significantly related to cardiovascular death among patients with documented CAD.17 Although their pathological role is unclear, elevated concentrations of soluble adhesion molecules have been found in patients with stable angina and acute coronary syndrome,18 a frequent cause of morbidity and mortality in patients with CAD.

Most patients with CAD have systemic atherosclerotic changes and it is well-known that hemodialysis (HD) patients also often have the complication of systemic and coronary atherosclerosis. End-stage renal disease is associated with a higher prevalence of several traditional and uremia-related risk factors for atherogenesis, such as hypertension (HT), hyperlipidemia (HL), diabetes mellitus (DM), hemodynamic overload, anemia and increased oxidative stress.19 In fact, a marked increase in the incidence of CAD and death rates has been reported in HD patients compared with an age-matched general population and to patients with hyperlipidemia and hypertension.20

Therefore, in the present study, we explored the possibility that the levels of expression of leukocyte adhesion molecules CD11a, CD18, ICAM-1, VLA-4 and L-selectin may be related to CAD or HD.

Methods

Study Population The study population included 55 patients who under-
went coronary angiography at the time of enrollment (20 who had initial coronary angiograms and significant coronary stenosis (defined as ≥50% luminal narrowing) and diagnosed with CAD (CAD group). 20 who had initial coronary angiograms but were without significant coronary stenosis (CONT group), and 15 who had undergone percutaneous coronary intervention 6 months previously and a restenosed coronary vessel was found on their second coronary angiogram (RESTE group). In addition, we also included 20 patients who were undergoing maintenance HD because of end-stage renal disease (HD group). Half of the patients in the HD group were diagnosed as diabetic nephropathy and the rest were chronic glomerular nephritis. Exclusion criteria were acute MI within the past 4 weeks, surgery or trauma within the past month, known malignant diseases, and febrile conditions. Patients with total cholesterol (TC) >220 mg/dl or triglyceride >150 mg/dl were considered to have hyperlipidemia (HL). Patients with systolic or diastolic blood pressure >160 mmHg or >95 mmHg, respectively, or who were under antihypertensive treatment were considered to have hypertension (HT). Patients who were being treated for DM or who had symptoms of DM were analyzed using commercially available statistical software (Statview-J 5.0; Abacus Concepts Inc, Calabasus, CA, USA).

### Results

**Patient Characteristics**

Table 1 shows the baseline patient characteristics in the 4 groups. Although there were no differences among the groups with regard to the prevalence of HT, DM or HL, the HD group included younger patients, a lower percentage of smokers, and a lower body mass index compared with the CONT group. Although patients had been treated with various combinations of medication, there was no significant difference in the drugs administered, such as angiotensin-converting enzyme, angiotensin-II type 1 receptor antagonists, calcium antagonists, β-blockers, long-acting nitrates, and HMG-CoA inhibitors in the CAD and RESTE groups.

### Expression of Monocyte Surface Adhesion Molecules

As shown in Fig 2, the CD11a and ICAM-1 expression on monocytes, but not lymphocytes or neutrophils, in the CAD group were significantly higher than in the CONT group. These expressions in the HD and RESTE groups
were similar to those in the CONT group. Interestingly, although monocyte CD11a and ICAM-1 expression in the CAD group were significantly higher than in the CONT group, these expressions in the RESTE group were comparable to those in the CONT group.

L-selectin expression on monocytes in the CAD group was also significantly higher than that in the CONT group, but there were no significant differences in CD18 or VLA-4 expression among the groups (Fig 2).

**Fig 1.** Representative results of flow cytometry to show the distribution of leukocytes (A), and monocyte IgG expression as a negative control (open area) and CD11a expression (shaded area) in a patient in the CAD group (B). Expression levels of adhesion molecules are shown as arbitrary units, and were calculated as [average channel of part (a)] x number of monocytes.

**Fig 2.** Expression levels of adhesion molecules, (a) CD11a, (b) ICAM-1, (c) CD18, (d) L-selectin and (e) VLA-4 on monocytes in patients without coronary stenosis (CONT group), with initially diagnosed CAD (CAD group), with restenosis (RESTE group) and undergoing HD (HD group). The levels of expression of CD11a and ICAM-1 in the CAD group were significantly higher than those in the CONT group. The expression of these molecules in the RESTE group and HD group were comparable to those in the CONT group. L-Selectin expression in the CAD group was also significantly higher than that in the CONT group. *p<0.05 vs CONT group.
Discussion

The main finding of the present study is that patients with initially diagnosed CAD had increased levels of expression of CD11a and ICAM-1 on monocytes. These findings may be important with regard to the potential role of leukocytes in the pathogenesis of atherothrombosis. In fact, leukocyte activation has been associated with increased adherence to the endothelium, increased leukocyte aggregation, and capillary plugging as well as increased oxidative burst and tissue damage. Thus, the finding of activated leukocytes in patients with CAD is consistent with modern theories regarding the possible detrimental effect that the inflammatory process might have on the development and prognosis of the initial stage of atherosclerosis.

Another important finding is that the levels of expression of CD11a and ICAM-1 on monocytes in patients with restenosed vessels were comparable to those in the CONT group. Although intracoronary stenting has reduced the rate of stenosis, in-stent restenosis, which is almost exclusively caused by neointimal hyperplasia, is still a major clinical problem. Because restenosis is caused by neointimal hyperplasia, the mechanism of stenosis in the CAD group is likely different from that in the RESTE group. Yasukawa et al reported that balloon injury of rat carotid arteries resulted in the strong expression of ICAM-1 on medial smooth muscle cells after 1 and 2 days, and this expression decreased after 5 or 7 days. The expression of ICAM-1 on neointimal smooth muscle cells and regenerating endothelial cells was observed later, at 5 or 7 days after injury. The increased expression of ICAM-1 may be important for inducing restenosis as an initial step after coronary intervention. Because we measured the ICAM-1 level 6 months after coronary intervention in this study, the ICAM-1 level in the RESTE group may return to that in the CONT group.

Treatment of animals with an antibody against ICAM-1 for 6 days resulted in a significant reduction in neointima formation, with no reduction in mononuclear cell infiltration. Although an antibody against CD11a had no effect, suggesting that some CD11a-independent function of ICAM-1 induced neointima proliferation, our findings suggest that some CD11a-dependent function(s) of ICAM-1 may promote the initial stenosis of vessels in humans. Our data are partially consistent with those of Yasukawa et al, and the time-course of treatment suggests that ICAM-1 expression on medial smooth muscle cells is a primary target of the beneficial effects achieved by antibody treatment.

In the present study, the levels of expression of monocyte CD11a/CD18 and ICAM-1 in HD patients were similar to those in patients without CAD. In another study, high plasma concentrations of soluble ICAM-1 were observed in HD patients compared with controls. Although these findings may be confirmed by further studies, several theories can be proposed. First, the levels of C-reactive protein in the previous study may have been higher than those in normal subjects. Second, the mechanism of atherosclerosis in HD patients may differ from that in CAD. Fewer biocompatible membranes in dialysis patients leads to a chronic activation of leukocytes, overproduction of proinflammatory cytokines, a sustained production of reactive oxygen/nitrogen species and consumption of antioxidant defenses, which subsequently leads to slow progress of atherosclerosis. Because these mechanisms are chronic, not acute reactions, it may be difficult to find the differences in the expression levels between HD patients and normal subjects.

The expression of L-selectin on monocytes in the CAD group was also significantly higher than that in the CONT group. Although CD11b/CD18 is upregulated and L-selectin is rapidly shed from the leukocyte surface by a proteolytic mechanism after leukocyte activation, an association between CD11a/CD18 and L-selectin has not been reported. Because soluble L-selectin inhibits the adhesion of leukocytes to cytokine-activated endothelial cells, the increased L-selectin expression observed in the present study may be caused by a compensatory effect against the increase in CD11a and ICAM-1-induced adherence.

Study Limitations

This study simply examined the association between adhesion molecules and coronary stenosis. A prospective trial in patients with initial stenosis or restenosis is needed to help demonstrate the potential effects of these adhesion molecules. Although we assessed coronary atherosclerosis by coronary angiography, intravascular ultrasonography should be used to detect significant atheroma in a future study.

Conclusion

We found that the monocyte expression of CD11a and ICAM-1 was normalized in patients with restenosed vessels, but was increased in patients with CAD. However, further studies are needed to complete our knowledge of their roles in the pathology of vascular disease and to promote the development of new therapeutic strategies.

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References


