Peripheral arterial disease (PAD), presenting as intermittent claudication, is one of the most common causes of pain and disability of the lower extremities. The incidence and prevalence of arteriosclerosis obliterans (ASO) increases with age in both males and females, whereas thromboangitis obliterans (TAO), or Buerger's disease, a nonatherosclerotic vascular occlusive disease, afflicts the peripheral arteries of young adult smokers. Both diseases are often characterized by an inexorable downhill course once the stage of critical limb ischemia associated with ulceration or gangrene is reached.

Recently, investigators have reported a relationship between cytokines and vascular disease. Vascular endothelial growth factor (VEGF) is a potent endothelial cell-specific mitogen that promotes angiogenesis and vasodilation, and thus the development of collateral vessels. Inoue et al reported that hypercellular and atheromatous lesions in human coronary arteries showed distinct VEGF positivity of activated endothelial cells, macrophages, and partially differentiated smooth muscle cells. Raised serum concentrations of VEGF have been described in acute coronary syndrome; and it is believed that VEGF production is an early adaptation of tissues to ischemia, enhancing collateral blood vessel formation. Using VEGF cDNA transfer, this concept has been validated in animal experiments and clinical investigations are under way.

Macrophage colony-stimulating factor (M-CSF) is a cytokine that stimulates the survival, proliferation and differentiation of progenitor cells of the monocyte/macrophage lineage and activates several functions of mature macrophages. Rosenfeld et al reported that M-CSF mRNA and protein were expressed in human atheromatous plaques; and Saitoh et al found that an increased concentration of circulating M-CSF reflected atherosclerotic progression and predicted future cardiac events in patients with coronary artery disease. We also reported that elevated concentrations of serum M-CSF predicted restenosis after percutaneous coronary intervention. The purpose of the present study was to clarify the roles of VEGF and M-CSF in the development of peripheral artery disease.

Methods

Subjects

We enrolled 10 patients with ASO, 10 with TAO and 10 healthy control subjects (5 male, 5 female) (Table 1). All the patients had a history of intermittent claudication, had previously undergone angiography of the lower extremities, and had had the lesions radiologically diagnosed. All the patients gave written informed consent to participate in this study.

Assays

Venous blood samples were obtained from the patients and control subjects, centrifuged and then stored at -80°C.
Cytokines and PAD

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Table 1 Patient Characteristics

<table>
<thead>
<tr>
<th>TAO (n=10)</th>
<th>ASO (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49±4</td>
<td>71±2</td>
</tr>
<tr>
<td>M/F</td>
<td>7/3</td>
<td>9/1</td>
</tr>
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<tr>
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<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig 1. Serum VEGF concentrations in patients with PAD. The VEGF concentrations in the ASO and TAO patients were significantly higher than those in the control subjects. There was no significant difference in the VEGF concentrations between the ASO and TAO patients. Data are presented as medians, with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (bars).

Statistics

Age was expressed as mean±SEM. Because the serum VEGF and M-CSF concentrations were nonparametrically distributed, these parameters were expressed as the median (interquartile range (IQR)). Comparisons between the patients and control subjects were performed using the unpaired t test and the Mann-Whitney U test as appropriate. Correlations were performed using the Spearman’s rank correlation method. A p<0.05 was considered significant.

Results

As expected, extensive differences in the risk factor profiles were observed between the ASO and TAO patients (Table 1). The mean age was significantly higher in the ASO patients than in the TAO patients, and hypertension and diabetes mellitus were more frequent complications in the ASO patients.

As shown in Fig 1, the serum VEGF concentrations in patients with ASO (median, 594; IQR, 463–948 pg/ml) and TAO (329; 261–602 pg/ml) were significantly higher than those in the control subjects (95; 26–190 pg/ml). There was no significant difference between the ASO and TAO patients in the VEGF concentration.

In contrast, the serum M-CSF concentrations in the ASO patients (871; 694–1,310 pg/ml) were significantly higher than those in the TAO patients (458; 330–685 pg/ml) and control subjects (367; 236–561 pg/ml), whereas there were no differences in the M-CSF concentrations between the TAO patients and control subjects (Fig 2).

Fig 3 shows that there was not a significant correlation between the serum VEGF and M-CSF concentrations in the patients and control subjects. The M-CSF concentrations did not correlate with those of VEGF. (Closed circles) ASO patients, (open circles) TAO patients, (closed triangles) control subjects.

Discussion

In the present study, we detected a significant increase in the serum VEGF concentrations in patients with PAD, as previously reported by other investigators.12,13 The increased concentration of VEGF in PAD may be the result of ischemia of the tissues on the distal to arterial obstructive lesions, because the expression of VEGF is strongly enhanced by tissue ischemia.14 Roller et al reported that the VEGF concentration increased in the plasma of patients with PAD, especially those in the advanced stage, also...
suggesting that severe ischemia induces the production of VEGF. In response to ischemic changes, angiogenesis may be driven by increased concentrations of VEGF, itself produced by the endothelium, platelets and smooth muscle cells, all of which are likely to be involved in the pathogenesis of PAD.

Celletti et al reported that recombinant VEGF delivered intraperitoneally to cholesterol-fed mice and rabbits increased the area of atherosclerotic plaques. The increased number of vasa vorum in the media of atherosclerotic arteries may reflect increased angiogenesis, and the present finding of high serum VEGF concentrations in ASO patients could be related to this process. The present study design did not allow us to determine whether the increased VEGF concentration in ASO is a cause or a consequence of the lesions, but a similar increase in the VEGF concentration in TAO, a nonatherosclerotic vascular disease, suggests the latter mechanism.

M-CSF specifically promotes growth and differentiation of the monocyte-macrophage lineage and could play an important role in the pathogenesis of atherosclerosis. Mozes et al demonstrated that gene transfer of cDNA encoding M-CSF induced local infiltration of macrophages and smooth muscle cells in the rabbit artery 5 days after treatment and recently, Murayama et al administered a monoclonal antibody against c-fms, the receptor of M-CSF, to apolipoprotein E-deficient mice and found that blockade of the M-CSF/c-fms pathway protected against early atherogenesis. Increased concentration of circulating M-CSF reflects atherosclerotic progression and also predicts future cardiac events in patients with angina pectoris. However, no data are available on the concentration of M-CSF in PAD. In the present study, we demonstrated a significant increase in the serum concentration of M-CSF in the ASO patients, but not in the TAO patients.

As shown in Fig 3, the serum concentration of M-CSF increased independently of that of VEGF, suggesting that the increased M-CSF concentration in ASO is not related to tissue ischemia. The difference between the ASO and TAO patients in the serum concentration of M-CSF may reflect the histopathological characteristics. TAO is a non-atherosclerotic segmental inflammatory disease that most commonly afflicts the small arteries and veins of the arms and legs. Pathologically, there is a highly cellular and inflammatory thrombus with relative sparing of the blood vessel wall. On the other hand, blood monocytes are the precursors of the lipid-laden foam cells that are the hallmark of the plaque lesions of ASO, and M-CSF may play an important role in their recruitment to the vessel wall. Recruitment of monocytes does not contribute to the pathogenesis of TAO.

In the present study, we for the first time demonstrated different concentrations of circulating VEGF and M-CSF in ASO and TAO patients, which may be related to the different pathogenesis of these vascular diseases. However, there was a limited number of patients in the present study and further investigation should be performed to elucidate the detailed mechanism of such increases in the cytokine concentrations in ASO and TAO.

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References