Resting and activated endothelial cells are increased in the peripheral blood of cancer patients

Patrizia Mancuso, Alessandra Burlini, Giancarlo Pruneri, Aron Goldhirsch, Giovanni Martinelli, and Francesco Bertolini

Circulating endothelial cells (CECs) were enumerated in 20 healthy controls and 76 newly diagnosed cancer patients by means of 4-color flow cytometry. In breast cancer (n = 46) and lymphoma (n = 30) patients, both resting and activated CECs were increased by 5-fold (P < .0008 vs control). CECs significantly correlated with plasma levels of vascular cell adhesion molecule-1 and vascular endothelial growth factor. Resting and activated CECs were similar to healthy controls in 7 lymphoma patients achieving complete remission after chemotherapy, and activated CECs were found to decrease in 13 breast cancer patients evaluated before and 24 hours after quadrantectomy. (Blood. 2001;97:3658-3661)

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Figure 1. Four-color flow cytometry evaluation of circulating endothelial cells and endothelial progenitors. (A) Representative panel (left) shows the analysis gate used to exclude platelets, death cells, and debris and the reference beads used to obtain absolute cell count. The other panel (right) shows the gate used to exclude hematopoietic cells expressing the CD45 antigen. (B,C) Middle and bottom panels indicate negative controls and the expression of antigens used to evaluate resting (CD31 and CD34), activated (CD105 and CD106), and progenitor (CD133) endothelial cells. (C) Bottom panels show the more activated phenotype of a representative newly diagnosed BC patient. R4 indicates CD45-hematopoietic progenitors depicted by high CD34 expression.

Results and discussion

In healthy controls (n = 20), mean values of resting and activated CECs were 7.9/µL (95% confidence interval [CI], 4.7-11.1) and 1.2/µL (95% CI, 0.1-2.3), respectively. Seven female controls were reevaluated during the menstrual period associated with physiological active angiogenesis. Mean activated CECs were found to increase from 2.4/µL (95% CI, <0.1-5.5) to 4.4/µL (95% CI, <0.1-10.5). However, this trend did not reach statistical significance (P = .15 by Wilcoxon matched-pairs test).

In 76 newly diagnosed patients, mean resting and activated CECs were 39.1/µL (95% CI, 16.8-61.4) and 6.8/µL (95% CI, 5.0-8.6), ie, increased by 5-fold (P < .0008 vs control by ANOVA). CEC distribution was normal in controls and skewed to the right in patients. Three of 5 lymphoma patients in leukemic phase contributed to most of the skewing observed in CEC distribution among patients; and lymphoma patients, compared with BC patients, had higher mean resting (78.0/µL [95% CI, 22.8-133.5]) vs 15.8/µL [95% CI, 12.0-19.7], P = 0.0078 by ANOVA) and activated (8.7/µL [95% CI, 5.1-12.3]) vs 5.6/µL [95% CI, 3.7-7.4], P = 0.18) CECs. Differences between BC patients with early or metastatic diseases were not significant. In controls and patients, the number of CECs did not significantly increase with age. Studies are ongoing to fully understand the clinical features associated with the particularly elevated CEC values observed in some patients, and repeated CEC measurements in patients and controls indicated a low longitudinal CEC variation. Evaluation of CD36 expression showed that in patients and controls at least half of the CECs were microvascular in origin.1

In patients and controls, the count of resting and activated CECs did not correlate with the count of white cells, red cells, or platelets.

VEGF is produced by most tumor cells7,8 and is involved in CEP mobilization.17 Mean VEGF was 83 pg/mL (95% CI, 27-140) and 192 pg/mL (95% CI, 103-281) in controls and patients, respectively (P = .03 by ANOVA). Correlation between MVD and VEGF and between MVD and CECs did not reach statistical significance. On the other hand, a positive correlation (r = 0.419, P = .009 by multiple regression) was found between CECs per microliter and plasma VEGF. As shown in Figure 2, a normal distribution of resting CECs, activated CECs, and plasma VEGF was observed in controls, whereas a switch to increased VEGF, increased CECs, and activated CEC phenotype was observed in cancer patients. Circulating VCAM-1, a glycoprotein produced by angiogenic endothelial cells, was significantly increased in patients

Circulating vascular endothelial growth factor (VEGF) and VCAM-1 were measured in the plasma of patients and controls by commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, and Biosource, Camarillo, CA, respectively) as we previously described.15 It has been suggested in the past that VEGF measurements may influence platelet shape, but no statistically significant difference in VEGF levels is observed in plasma samples collected with EDTA versus platelet stabilization.

Table 1. Target antigens, related cluster designations, antibody clones, and conjugation of monoclonal antibodies used in the study

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cluster designation</th>
<th>Antibody clone</th>
<th>Conjugation</th>
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<tbody>
<tr>
<td>PECAM-1</td>
<td>CD31</td>
<td>WM59*</td>
<td>FITC</td>
</tr>
<tr>
<td>Glycoprotein</td>
<td>CD34</td>
<td>HPCA-2†</td>
<td>APC</td>
</tr>
<tr>
<td>LCA</td>
<td>CD45</td>
<td>2D1†</td>
<td>PerCP</td>
</tr>
<tr>
<td>Endoglin</td>
<td>CD105</td>
<td>8E11‡</td>
<td>FITC</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>CD106</td>
<td>5110C9*</td>
<td>PE</td>
</tr>
<tr>
<td>AC133</td>
<td>CD133</td>
<td>AC133/1§</td>
<td>PE</td>
</tr>
<tr>
<td>P1H12</td>
<td>Not yet designated</td>
<td>P1H12†</td>
<td>FITC</td>
</tr>
</tbody>
</table>

PECAM-1 indicates platelet endothelial cell adhesion molecule-1; LCA, leucocyte common antigen; VCAM-1, vascular cell adhesion molecule-1; FITC, fluorescein isothiocyanate; APC, allophycocyanin; PerCP, peridinin chlorophyll protein; PE, R-phycocerythrin.

*Products were supplied by BD Pharmingen (San Diego, CA).
†Products were supplied by BD (Mountain View, CA).
‡Products were supplied by Euroclone (Devon, United Kingdom).
§Products were supplied by Miltenyi (Bergisch Gladbach, Germany).
[Products were supplied by Chemicon (Temecula, CA).]
pared with those with BC ($P < .002$). These patients, mean resting and activated CECs were 12.8/μL (95% CI, 0.4-1.2), respectively.

CECs were found to be similar to control values in 7 lymphoma patients who achieved complete remission after chemotherapy. In parallel, the measurement of CEC viability is currently under investigation to ascertain whether a given drug therapy has antiangiogenic properties.

**Note added in proof.** Chang et al. have recently provided evidence indicating that tumor blood vessels may be mosaics in which both endothelial and tumor cells form the luminal surface. These data provide a possible explanation for the finding of high CEC numbers in cancer patients.

### References

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