The association of ICAM-1 Exon 6 (E469K) but not of ICAM-1 Exon 4 (G241R) and PECAM-1 Exon 3 (L125V) polymorphisms with the development of differentiation syndrome in acute promyelocytic leukemia

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Abstract: The use of all trans-retinoic acid (ATRA) is the basis of treatment of acute promyelocytic leukemia (APL) and represents the paradigm of differentiation therapy. In general, ATRA is well-tolerated but may be associated with a potentially lethal side-effect, referred to as retinoic acid or differentiation syndrome (DS). The cellular and molecular mechanisms of DS are poorly understood and involve changes in the adhesive qualities and cytokine secretion of leukemic cells during ATRA-induced differentiation. As leukocyte extravasation is a key event in DS pathogenesis, we analyzed the association between the polymorphisms at Exon 4 (G241R) and Exon 6 (E469K) of ICAM-1 and Exon 3 (L125V) of PECAM-1 genes with DS development in APL patients treated with ATRA and anthracyclines. DS was diagnosed in 23/127 (18.1%) APL patients at an average of 11.5 days after the start of ATRA. All patients presented respiratory distress associated with increased ground-glass opacity in chest radiographies. Other accompanying symptoms were: fever not attributable to infection (65.2%), generalized edema (37.5%), weight gain (37.5%), and impairment of renal function (8.6%). We detected an association between development of DS and the AA genotype at Codon 469 of ICAM-1 (odds ratio of 3.5; 95% confidence interval: 1.2–10.2). Conversely, no significant association was detected between G241R or L125V polymorphisms at Exon 4 of ICAM-1 and Exon 3 of PECAM-1, respectively. Our results suggest that susceptibility to DS in APL patients may be influenced by genetic variation in adhesion molecule loci. J. Leukoc. Biol. 82: 1340–1343; 2007.

Key Words: acute myelogenous leukemia • all trans-retinoic acid • adhesion molecules

Acute promyelocytic leukemia (APL) is invariably associated with gene rearrangements involving the retinoic acid receptor α (RARα) locus on Chromosome 17. In the majority of cases, RARα is translocated and fused to the promyelocytic leukemia (PML) gene on Chromosome 15 as a consequence of the t(15;17)(q22;q21). Morphologically, two variants of APL are recognized: the granular and the hypogranular, corresponding to the M3 and M3v subtypes of the FAB classification, respectively. APL treatment is based on the use of all trans-retinoic acid (ATRA), which induces terminal granulocytic differentiation of blasts and clinical remission. ATRA is generally well-tolerated but may be associated with a potentially lethal side-effect, referred to as RA or differentiation syndrome (DS) [1, 2]. Initially described by Frankel et al. [3], this syndrome is characterized by fever, pleural and pericardial effusion, respiratory distress, weight gain, and pulmonary infiltrates noted on chest radiography. Six percent to 27% of APL patients develop DS, and mortality rates range from 1% to 7% [4–6]. Previously reported, predictive factors for development of DS include high leukocyte counts at diagnosis, hypogranular variant, PML/RARα bcr3 isoform, and a pattern of CD13 and CD33 expression [1, 2, 6–8]; however, the existence of these associations is controversial.

The cellular and molecular mechanisms of DS are poorly understood, and the proposed mechanisms involve changes in the adhesive qualities and cytokine secretion during ATRA-induced differentiation [9]. The ICAM-1 (CD54) and PECAM-1 (CD31) are members of the Ig superfamily. ICAM-1 is widely expressed at basal levels on the surface of endothelial cells and leukocytes [10] and in the APL cell line NB4 [11] and can be up-regulated by proinflammatory cytokines and by...
respiratory distress, and suggestive pulmonary infiltrates were
detected in 14 patients. No nodule formation nor septal lines
developed in patients at an average of 11.5 days (between the
4th and 20th days) after the start of ATRA, and other accom-
panying symptoms were: fever not attributable to infection
(65.2%), generalized edema (37.5%), weight gain (37.5%), and
impairment of renal function (8.6%). Table 1 presents clinical
and laboratorial features at diagnosis of APL patients, with and
without DS. No significant differences were detected between
the two groups. It is worthwhile to point out that WBC counts
and the frequency of cases presenting with more than 10,000
leukocytes/μl, were not significantly higher in the group of
patients with DS. Our results corroborate those by de Botton et al. [2] and Vahdat et al. [6], suggesting that high WBC counts
are not a predictive factor for DS development. Chest radi-
ographs of all patients showed increased ground-glass opacity
and an increased vascular pedicle width. Pleural effusion was
detected in 14 patients. No nodule formation nor septal lines
were observed. These findings are consistent with those of Jung
et al. [17].

The genotype and allele frequencies for ICAM-1 and PE-
CAM-1 polymorphisms were shown in Table 2. The genotype
frequencies did not deviate significantly from that predicted,
based on the Hardy-Weinberg equilibrium. Compared with

### Table 1. Demographics and Complete Blood Counts of APL Patients with or without DS at Diagnosis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DS (n=11)</th>
<th>No DS (n=59)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>31.05 (±19.05)</td>
<td>33.65 (±16.71)</td>
<td>0.455</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>11/12</td>
<td>59/45</td>
<td>1.000</td>
</tr>
<tr>
<td>WBC counts (μl)</td>
<td>30,734 (±61,152)</td>
<td>15,994 (±29,247)</td>
<td>0.194</td>
</tr>
<tr>
<td>Cases with WBC &gt; 10,000/μl</td>
<td>8 (36.4%)</td>
<td>29 (29.3%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.38 (±2.26)</td>
<td>8.42 (±2.64)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Platelets (μl)</td>
<td>19,690 (±15,610)</td>
<td>24,650 (±31,480)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* Values presented as mean (±SD); Hb, Hemoglobin.

### Table 2. Genotype and Allele Distribution of the Polymorphisms Analyzed in APL Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>APL patients</th>
<th>Controls</th>
<th>P value</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 Exon 4 (G241R)</td>
<td>GG</td>
<td>96 (87.3%)</td>
<td>100 (93.5%)</td>
<td>0.213</td>
<td>92.7:7.3</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>12 (10.9%)</td>
<td>7 (6.5%)</td>
<td>0.168</td>
<td>G:G</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>2 (1.8%)</td>
<td>0 (0)</td>
<td>OR: 2.1</td>
<td>96.7:3.3</td>
</tr>
<tr>
<td>ICAM-1 Exon 6 (E469K)</td>
<td>GG</td>
<td>41 (33%)</td>
<td>20 (8%)</td>
<td>OR: 0.0001</td>
<td>G:G</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>63 (50.3%)</td>
<td>136 (54.6%)</td>
<td>0.1</td>
<td>58.5:41.5</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>20 (16.2%)</td>
<td>93 (37.4%)</td>
<td>0.1</td>
<td>35.3:64.7</td>
</tr>
<tr>
<td>PECAM-1 Exon 3 (L125V)</td>
<td>CC</td>
<td>13 (10.2%)</td>
<td>55 (22.3%)</td>
<td>0.213</td>
<td>40.5:59.5</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>77 (60.6%)</td>
<td>141 (57%)</td>
<td>0.504</td>
<td>C:G</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>37 (29.2%)</td>
<td>51 (20.7%)</td>
<td>OR: 2.5</td>
<td>50.8:49.2</td>
</tr>
</tbody>
</table>

* Data presented as n (%). OR, Odds ratio (95% confidence interval). * For comparison between GG and GA + AA frequencies. 5 For comparison between GG and GA + AA frequencies. 6 For comparison between GG and GA + AA frequencies.
controls, APL patients presented a higher frequency of GG genotype at Exon 6 of ICAM-1 and a lower frequency of CC at Exon 3 of the PECAM-1 gene. The observed frequency of G allele at ICAM-1 Exon 6 was higher than reported by Amodu et al. [18] in an African population and lower than that described by Ghadegesin et al. [15] and by Jiang et al. [19] in English and German populations, respectively, probably reflecting the African contribution to the formation of the Brazilian population. In contrast, little difference was observed concerning ICAM-1 Exon 4 [15, 16] and PECAM-1 [15, 20] polymorphisms.

We detected an association between development of DS and the AA genotype at Codon 469 of ICAM-1 (odds ratio of 3.5; 95% confidence interval: 1.2–10.2; Table 3). There was no significant association between ICAM-1 G241R or PECAM-1 E469K polymorphisms and DS. However, one should bear in mind that a sample size of 12,000 subjects in each group would be necessary for detecting the observed risk estimated for PECAM-1 polymorphism at 80% power with an α of 0.05. The samples sizes needed for the observed risk estimated for ICAM-1 G241R and E469K polymorphisms at the same test conditions would be 400 and 70 subjects in each group, respectively. It is unclear how the E469K polymorphism may affect ICAM-1 multiple functions. Nevertheless, Amodu et al. [18] demonstrated that the presence of the G allele at position 469 was associated with increased risk (3.6 times) of severe promyelocytic leukemia who developed the retinoic acid syndrome. Blood 95, 90–95.


