To the editor:

Discrepancy between phenotype and genotype on screening for factor V Leiden after transplantation

Factor V (FV) Leiden is the most common genetic defect found in persons of European descent with venous thromboembolism.1 The 1691G>A transition determines the R506Q substitution.2 This suppresses a cleavage site for activated protein C (APC) on FVa, resulting in resistance to APC in a functional coagulation-based assay.3 FV Leiden accounts for over 90% cases of APC resistance, but this phenomenon may also be associated with pregnancy, oral contraceptive use, lupus anticoagulant, elevated FVIII levels, and rarely mutations other than FV Leiden.4,5 Although APC resistance may occur in the absence of FV Leiden, the converse is usually not true. We describe 2 patients who, following bone marrow and liver transplantation respectively, developed anomalies between APC resistance and FV Leiden genotype.

A 38-year-old female presented with a right-sided iliofemoral deep-vein thrombosis (DVT) secondary to venous obstruction by inguinal lymphadenopathy. She was diagnosed to have stage IV low-grade follicular B-cell non-Hodgkin lymphoma (NHL) and received combination chemotherapy. After a second relapse, she underwent sibling allogeneic progenitor cell transplantation with busulphan (16 mg/kg) and cyclophosphamide (200 mg/kg) conditioning. After transplantation, she developed menopausal symptoms and was considered for hormone-replacement therapy (HRT). Further questioning revealed that, although her stem cell donor was well, her other sibling had previously suffered a spontaneous popliteal DVT. In view of this history and the 2- to 4-fold increased risk of venous thrombosis associated with HRT,4 thrombophilia screening was undertaken. Resistance to APC in FV-deficient plasma was normal. FV Leiden genotyping by PCR and Hind III restriction enzyme analysis, however, demonstrated heterozygosity for FV Leiden (G/A). In view of these unexpected results, mutation analysis of stored pretransplantation DNA was performed. Because this revealed a normal FV genotype (G/G), we surmised that the mutant FV giving rise to APC resistance originated from the donor liver.

Table 1. APC-sensitivity ratios and Factor V Leiden genotype after tissue transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>APC-sr (normal range 2.0-2.8)</th>
<th>FV Leiden genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1:</td>
<td></td>
<td></td>
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<tr>
<td>Stem-cell recipient</td>
<td></td>
<td></td>
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<tr>
<td>Pretransplantation</td>
<td>—</td>
<td>G/G</td>
</tr>
<tr>
<td>Posttransplantation</td>
<td>2.7</td>
<td>G/A</td>
</tr>
<tr>
<td>Sibling 1 (stem-cell donor)</td>
<td>1.7</td>
<td>G/A</td>
</tr>
<tr>
<td>Sibling 2 (spontaneous DVT)</td>
<td>1.6</td>
<td>G/A</td>
</tr>
<tr>
<td>Case 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver recipient (posttransplantation)</td>
<td>1.5</td>
<td>G/G</td>
</tr>
<tr>
<td>Donor-liver biopsy</td>
<td>—</td>
<td>G/A</td>
</tr>
</tbody>
</table>

The second patient was a 41-year-old female who had undergone orthotopic liver transplantation (OLT) for cirrhosis secondary to Wilson disease. She became pregnant 1 year after OLT. At 20 weeks’ gestation she developed progressive hepatocellular failure, and thrombophilia screening was performed to investigate possible hepatic vaso-occlusion. Although her APC-sensitivity ratio (in FV-deficient plasma) of 1.5 would have been consistent with heterozygosity for FV Leiden, genotyping was normal. But DNA analysis is routinely performed on peripheral blood leukocytes, which may not be representative of donor liver genotype. Analysis of donor-liver biopsy samples for FV Leiden demonstrated heterozygosity (G/A), confirming that the mutant FV giving rise to APC resistance originated from the donor liver.

The FV Leiden mutation results in resistance to APC and may be associated with a 3- to 8-fold increased risk of venous thrombosis in heterozygotes and an 80-fold increased risk in homozygotes.2 Screening for FV Leiden involves a combination of coagulation and genetic assays. The conventional APC-resistance assay will detect not only mutant factor V but also several conditions associated with the APC-resistant phenotype that may also pose a thrombotic risk. These conditions may be excluded by use of the modified APC-resistance assay in which samples are prediluted in FV-deficient plasma.7 Confirmatory genetic analysis usually employs DNA extracted from peripheral blood leukocytes. Although these would have the same genotype as hepatic tissue in normal subjects, this might not be the case after transplantation. We highlight this possible discrepancy between genotype and phenotype also previously reported in 2 subjects who, in a reversal of the situation described here, were heterozygous for FV Leiden and received bone marrow and liver transplants, respectively, from FV wild-type donors.8

In humans, circulating FV is synthesized primarily by hepatocytes with a smaller pool originating from megakaryocytes.8,9 In our first patient, circulating FV synthesized by recipient liver would be of wild type, despite heterozygosity for FV Leiden in donor-derived hematopoietic cells. The risk of venous thromboembolism would not, therefore, be significantly increased, and she was able to receive HRT. In contrast, production of mutant FV by donor hepatocytes indicates that the second patient might have an increased risk of thrombosis despite a normal FV Leiden genotype. We therefore elected to give her thromboprophylaxis in the postpartum period. These cases illustrate the difficulties of thrombophilia testing after tissue transplantation and emphasize the need for evaluating both FV phenotype and genotype in order to accurately assess thrombotic risk in such patients.

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References


To the editor:

Serum viral interleukin-6 in AIDS-related multicentric Castleman disease

Viral interleukin-6 (vIL-6) encoded by Kaposi sarcoma (KS)-associated herpesvirus (KSHV) has been detected in 3 acquired immunodeficiency syndrome (AIDS)-related disorders: KS, primary effusion lymphoma, and multicentric Castleman disease (MCD). Similar to its human counterpart, vIL-6 supports the growth and survival of certain mouse and human cell lines in vitro.

When expressed in mice, recombinant vIL-6 induced marked lymph-node plasmacytosis similar to morphologic changes seen in the plasma-cell variant of human MCD. MCD is a lymphoproliferative disorder featuring systemic manifestations of inflammation and B-cell hyperreactivity. Virtually all HIV-positive cases of MCD and nearly 50% of HIV-negative cases are infected with KSHV, and all KSHV-positive MCD tissues were found to express vIL-6. In contrast to localized Castleman disease that typically remits after surgical removal of the localized mass, the multicentric form, occurring in a small percentage of patients, behaves more aggressively and is frequently fatal. Immunohistochemical studies demonstrated abundant human IL-6 (hIL-6) expression in the germinal centers of MCD lymph nodes, and there is evidence for a pathogenetic role of hIL-6 in MCD. KSHV vIL-6 is expressed by immunoblastic cells present among the mantle zone of MCD. The expression of vIL-6 in MCD lesions and the functional similarities of cellular and vIL-6 raised the possibility that vIL-6 may also play a role in the pathogenesis of MCD. But vIL-6, if any, in MCD or other KSHV-associated disorders has not been fully examined. As a step toward further understanding a potential pathogenetic role of vIL-6, we measured circulating vIL-6 levels in an HIV-positive patient at the onset of MCD.

A 40-year-old HIV-positive homosexual man presented with a sore throat, nonproductive cough, night sweats, fever, diarrhea, marked fatigue, and weight loss in June 1999. The patient had received highly active antiretroviral therapy (HAART) since July 1996; including lamivudine (3TC; 150 mg × 2/d), stavudine (d4T; 40 mg × 2/d), and nelfinavir (NFV; 2250 mg × 1/d). HIV-RNA load had been stable at less than 1200 copies/mL since 1996. CD4+ T-cell counts had remained over 300 cells/mm³. On physical examination, the patient had lymphadenopathy, abdominal distention with hepatosplenomegaly, abdominal tenderness, and skin rash, but no evidence of KS. Oral administration of prednisone (30 mg/d) was started, to relieve systemic symptoms. A cervical lymph-node biopsy displayed the typical features of mixed plasma-cell/hyaline vascular type of MCD: concentric layers of small lymphocytes surrounding the germinal centers and plasma cell infiltration in the interfollicular areas (Figure 1A-B). KSHV infection in the patient was confirmed by immunohistochemical detection of KSHV–latency-associated nuclear antigen (KSHV-LANA) in a lymph-node biopsy specimen (Figure 1C). A vIL-6–specific monoclonal antibody detected vIL-6 expression in the same specimen by immunohistochemistry (Figure 1D). The KSHV-LANA–positive and vIL-6–positive cells, similar in frequency, localized predominantly to the mantle zone of the lymph node. By contrast, expression of hIL-6, which has previously been detected in the germinal centers of certain MCD cases, was not detectable by immunohistochemistry in this lymph node (data not shown). Due to a dramatic improvement of systemic symptoms, prednisone was tapered after 10 days, and administration of the antiretrovirus agent foscarnet (7g × 2/day) started, followed by splenectomy in September 1999. Fifteen months later, the patient continued to be well, in remission, on occasional maintenance chemotherapy.

Serum vIL-6 was initially detected at 4756 pg/mL (Figure 2). But vIL-6 decreased to undetectable (less than 300 pg/mL) levels over the next 10 days and subsequently remained undetectable. By contrast, serum levels of hIL-6 fluctuated at low levels (range, <1.0-10.8 pg/mL) throughout this period. The HIV-RNA load presented marked changes during this period. For approximately 3 years prior to the onset of MCD, the HIV-RNA load in this patient had remained at less than 1200 copies/mL. After MCD was diagnosed, the HIV-RNA load peaked at 146 460 copies/mL, followed by a rapid decrease to 792 copies/mL on day 21 of treatment (Figure 2). Of note, the patient continued to receive the...
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