mucosae with gross cervical lymphadenopathy appeared. These new lesions caused upper airway obstruction with respiratory distress that required a tracheotomy and hemiglossectomy. Biopsies of skin and mucosae confirmed Kaposi’s sarcoma. Serum immunofluorescence assay showed a high anti-human herpes virus-8 (HHV-8) IgG titer (1:2,560), which demonstrated recent or active HHV-8 infection.6 Genomic DNA was prepared from sections of paraffin-embedded blocks of the lesion. HHV-8 detection was performed by polymerase chain reaction (PCR) using primers KS1 and KS2.9 An amplification product of 233 bp was obtained in samples, indicating the presence of HHV-8 virus in neoplastic tissue. Immunosuppressive therapy was discontinued on day +479, but the lesions did not remit. The patient died from massive lung hemorrhage on day +486. Permission for post mortem examination was denied, and the cause of the lung hemorrhage could not be determined.

Kaposi’s sarcoma has a negligible incidence in the general population, but has been observed in up to 6% of patients undergoing organ transplantation. Moreover, the incidence of KS is higher in patients treated with CsA and some authors have suggested that this association might reflect a state of overimmunosuppression rather than a specific effect of the molecule.1-4 Some studies have found DNA sequences of viral agents in tissue samples of KS and suggest the existence of a new herpesvirus, the human herpesvirus-8 or Kaposi’s sarcoma-associated herpesvirus.4,6,9,10 The high anti-HHV-8 IgG titer and the presence of HHV-8 virus in neoplastic tissue of our patient demonstrated recent HHV-8 IgG titer and the presence of HHV-8 virus in neoplastic tissue samples of KS and suggest the existence of a new herpesvirus, the human herpesvirus-8 or Kaposi’s sarcoma-associated herpesvirus.4,6,9,10 The high anti-HHV-8 IgG titer and the presence of HHV-8 virus in neoplastic tissue of our patient demonstrated recent or active HHV-8 infection.

In our view, prolonged therapy with CsA and other immunosuppressive drugs and the immune deficiency associated with severe chronic GVHD may have predisposed to the development of KS in the case described here.

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Key words
Kaposi’s sarcoma, sickle cell disease, bone marrow transplantation, graft-vs-host disease, immunosuppression.

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References

Platelet cryopreservation using second-messenger effectors and low-dose (2%) dimethyl sulfoxide. In vitro evaluation of post-thawing platelet activity with the platelet function analyzer

Sir,

At present, 5-6% DM SO is considered the most effective cryopreserving agent in terms of cell yield and residual function of recovered platelets.1 Recently, second messenger effectors (ThromboSol®), together with low-dose dimethyl sulfoxide (DMSO) (2%), are described as a new cryopreservation solution. The solution allows high yield recovery of cryopreserved platelets with a residual platelet function equal or superior to that of 6% DMSO-cryopreserved platelets. The hemostatic function of cryopreserved platelets was measured by PFA-100™.

Sir,

At present, 5-6% DM SO is considered the most effective cryopreserving agent in terms of cell yield and residual function of recovered platelets.1 Recently, second messenger effectors (ThromboSol®) (TC) have been used combined with low-dose (2%) DMSO, to cryopreserve platelet concentrates at ~80°C for clinical use.2,4 The aim of the present work was to compare the yield and function of platelets frozen with either 6% DMSO or TC-2% DM SO.

An intriguing problem is the in vivo measurement of objective parameters capable of predicting the in vivo hemostatic function of the recovered platelets. Mimicking physiologic platelet-subendothelial interactions, the platelet function analyzer (PFA100™) measures in vitro closure time, a surrogate index of in vivo bleeding time.5 Having previously developed a procedure to use PFA100 to assess platelet function in platelet concentrates,6 we used this procedure to compare residual platelet function in concentrates frozen using the two protocols. Yield, morphology and adhesion capacity were evaluated altogether. Thawed platelets were evaluated prior to and following a single washing procedure to remove about 95% of the TC and DM SO. Platelets were obtained by buffy-coat frac-
tion of whole blood units collected in CPDA.

ThromboSol® (TC) (LifeCell Corp. Branchburg, NJ, USA) in pure DMSO containing amiloride (Na⁺ H⁺ exchange inhibitor), adenosine, and sodium nitroprusside, which are cAMP and cGMP stimulators, respectively. TC was added (1:50 v/v) to the platelet suspension. After mixing, the suspension was placed at -80°C. Platelet bags were thawed by immersion into a 38°C water bath, under continuous agitation until complete dissolution of the solid phase. The 6% DMSO frozen platelets were obtained and thawed using standard protocols.

Platelet recovery was assessed by cytomteric platelet count (Sysmex K-800, Toa Medical Electronics, Kobe, Japan). Platelet morphology was evaluated using a phase-contrast microscope equipped with a 40X objective. The primary platelet hemostatic capacity (adhesion phase) was investigated by pipetting 50 µL of platelets (100x10⁹/L) over the surface of a cleaned 240x240 mm glass coverslip. After incubation in a moist chamber for 30 minutes, non-adherent cells were removed and the adherent cells were counted using a phase-contrast microscope equipped with a 40X objective. Platelet function was measured by PFA100™, following reconstitution of whole blood. Platelet function was defined as closure time (C-ADP or C-epinephrine, accordingly to the cartridge used) or as PFA-Index (PFA-I), calculated using the following formula:

\[
PFA-PI = \frac{\text{total flow volume (µL) : flow rate (µL/min)} \times \text{closure or elapsed time (sec)}}{100}
\]

After thawing and washing, platelet recovery, discoid platelets, and the absolute number (four fields) of glass-adherent cells were measured (Figure 1). The mean results were respectively 71, 69, and 179 for the TC-treated platelets and 57, 54, and 67 for the DMSO-treated platelets. The corresponding p values were: < 0.005, <0.001, and < 0.001.

The PFA100™ TM results, evaluated as C-ADP, C-epinephrine, and PFA-PI are reported in Table 1.

### Table 1. PFA100™ measurement of functional activity.

<table>
<thead>
<tr>
<th>Cartridge</th>
<th>ThromboSol-Treated Platelets</th>
<th>DMSO-Treated Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Unwashed</td>
<td>Washed</td>
</tr>
<tr>
<td></td>
<td>Thawed ADP epinephrine</td>
<td>ADP epinephrine</td>
</tr>
<tr>
<td>Closure Time (sec)</td>
<td>96.7 ±5.2 139.9 ±33.8</td>
<td>300³ 300⁴</td>
</tr>
<tr>
<td>PFA-Index</td>
<td>0.71 ±0.71</td>
<td>0.63 ±0.57</td>
</tr>
</tbody>
</table>

1Mean value ± standard deviation, n=8; ²p<0.005 for the values for cryopreserved samples as compared to the values for fresh samples. ³p>0.001 for the values for cryopreserved samples as compared to the values for fresh samples. ⁴p<0.05 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. ⁵p<0.005 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. ⁶p<0.01 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. ⁷p<0.05 for the values for cryopreserved samples as compared to the values for fresh samples. ⁸p<0.005 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. ⁹p>0.001 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. ¹⁰p<0.01 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. Comparison by the two-tailed paired t-test.
In conclusion, quantitative, morphologic and functional data, particularly when measured in vitro as PFA-PI using an ADP cartridge, showed that TC-cryopreserved platelets are at least as good as, and possibly superior to, DM SO-cryopreserved platelets.

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Key words
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References

Anti-D immunoglobulin in children with newly diagnosed immune thrombocytopenic purpura: a pilot study

Treatment options for childhood immune thrombocytopenic purpura (ITP) include observation, steroids, intravenous gammaglobulin (IVIG) and splenectomy. Recent studies²-⁴ have shown that anti-D increases the platelet count in children with ITP but that the time to achieve a platelet count ≥ 20,000/µL is significantly longer than following IVIG.² We gave anti-D as a single intravenous dose of 50 µg/kg to 10 consecutive Rh positive children with newly diagnosed ITP.

Sir,

Newly diagnosed patients aged 6 months to 18 years with a clinical diagnosis of ITP based on history, physical examination and isolated thrombocytopenia with a normal blood smear, were eligible for entry into the study. Initial evaluation consisted of a medical history and physical examination, complete blood count with differential, blood type and direct Coombs’ test. After informed consent had been obtained, all Rh positive, Coombs’ negative patients were treated with one dose of intravenous anti-D (Winrho-SD, Univax Biologics, Inc, Rockville, MD, USA) 50 µg/kg over 5 minutes. Complete blood counts were done every 12 hours until the platelet count was ≥ 20,000/µL at which point the patient was discharged home. Response was defined as an increase in platelet count to ≥ 20,000/µL with cessation of bleeding. This study was approved by the institutional review board at Baystate Medical Center, Springfield, Massachusetts.

The patients’ characteristics and results of treatment are presented in Table 1. Platelet counts for the first 40 hours post-anti-D are shown in Figure 1. All 10 patients responded to anti-D. The mean time to platelet count ≥ 20,000/µL was 22.3 ± 11.4 hrs (median 16.4 hrs; range 12-39.5 hrs). Median post-anti-D peak platelet count was 253,000/µL occurring at a median of 8 days post-treatment. The average drop in hemoglobin was 1.27 g/dL ± 0.7. Three patients were retreated for platelet counts < 20,000/µL. No allergic complications occurred, and no patients required transfusions secondary to anti-D-induced hemolysis. Two patients had mild headache 2-3 hours post-infusion; one also had emesis. All symptoms resolved with acetaminophen and diphenhydramine. Therapeutic options for childhood ITP remain controversial²-⁷ and include IVIG, corticosteroids, anti-D or less commonly splenectomy. Anti-D has potential advantages over IVIG: lower cost² and fewer side effects (headache, vomiting, sepsis, meningitis).³,⁴ The presumed method of action for anti-D is saturation of Fc receptors in the spleen with antibody-coated red blood cells.

Blanchette et al.⁵ randomized 146 children with ITP to receive low dose IVIG (0.8 g/kg), high dose IVIG (1 g/kg/day x2), intravenous anti-D (25 µg/kg/day x 2)