or prednisone (4 mg/kg/day orally, tapering off by
day 21). Both the low and high dose IVIG arms were
superior to anti-D in mean time to platelet count
> 20,000/µL: 1.4 versus 2.9 versus 3.9 days, respec-
tively. Tarantino et al.10 retrospectively compared chil-
dren receiving 0.8-1 g/kg IVIG (N=14) or 45-50 µg/kg
anti-D (N=13) and reported a mean time to platelet
count ≥20,000/µL of 1.26±0.82 days and 1.54±0.51
days. Although the number of patients in both our
study and the study by Tarantino is small, the use of
a single dose of 50 µg/kg rather than two daily dos-
eses of 25 µg/kg may have been the cause of the
improved response time.

In conclusion, a single 50 µg/kg intravenous dose
of anti-D produced a rapid increase in platelet count
in children with newly diagnosed acute ITP. A ran-
domized trial comparing higher doses of anti-D to
IVIG in children with acute ITP appears warranted.

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Key words
Immune thrombocytopenic purpura, anti-D, children

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References
1. Beardsley DS, Nathan DG. Platelet abnormalities in
infancy and childhood. In: Nathan DG, Oski FA, eds.
center study of the treatment of childhood chronic
idiopathic thrombocytopenic purpura with anti-D. J
3. Bussel JB, Graziano JN, Kimberly RP, Pahwa S, Ale-
dort LM. Intravenous anti-D treatment of immune
thrombocytopenic purpura: analysis of efficacy, toxic-
ity, and mechanism of effect. Blood 1991; 77:884-
93.
anti-D treatment of immune thrombocytopenic pur-
pura: experience in 272 patients. Blood 1997; 89:
2689-700.
trial of intravenous immunoglobulin G, intravenous
anti-D, and oral prednisone in childhood acute
immune thrombocytopenic purpura. Lancet 1994;
344:703-7.
globulin G therapy, oral prednisone therapy, and no
therapy in childhood acute immune thrombocy-
7. Sartorius JA. Steroid treatment of idiopathic throm-
buscoplastic purpura in children: preliminary results of
a randomized cooperative study. Am J Pediatr Hema-
8. [Anonymous]. Rho(D) immune globulin IV for pre-
vention of Rh isoimmunization and for treatment of
9. Kattamis AC, Shankar S, Cohen AR. Neurologic com-
plications of treatment of childhood acute immune
thrombocytopenic purpura with intravenously admin-
istered immunoglobulin G. J Pediatr 1997; 130:281-
3.
10. Tarantino MD, Madden RM, Fennewald DL, Patel CC.
Treatment of childhood acute immune thrombocy-
topenic purpura with anti-D immune globulin or

Treatment of refractory ITP with extracorporeal
immunoadsorption over a protein-A sepharose
column: a report of two cases

Two females with refractory ITP underwent plas-
ma immunoadsorption over protein-A sepharose
columns. The immediate response to immunoad-
sorption was unsuccessful while anti-platelet and
anti-HLA antibodies disappeared from serum.
However platelets progressively rose to normal in
the following months, medical therapy was gradu-
ally withdrawn and the patients remain in remis-
sion so far.

Sir,
Extracorporeal immunoadsorption of antibodies
over a protein A-silica matrix (Prosorba®, USA) has
been recently proposed among second line therapy
for refractory chronic immune thrombocytopenia
(ITP).2-5 Plasma immunoadsorption over protein A-
sepharose columns (Excorim/Citem 10 (EC10®),
Haematologica vol. 85(8):August 2000
Excorim, Lund, Sweden) is a two column system that allows the processing of larger amounts of plasma during each procedure as compared to Prosorba. While EC10 proved to be effective in the removal of acquired inhibitors to factor VIII or factor IX, there are no reports about refractory ITP.

Patient #1. A 67-year old female affected by chronic ITP was unsuccessfully given steroids, high dose immunoglobulins (IVIgG), danazol and two courses of vincristine. Only a transitory response occurred after splenectomy and symptomatic thrombocytopenia persisted despite treatment with plasmapheresis, azathioprine 100 mg/day, and then mesterolon 50 mg/day each. She was, therefore, offered experimental treatment with EC10. Her platelet count was 24×10^9/L, platelet-associated immunoglobulins (PAIgG) as demonstrated by direct immunofluorescence were increased and anti-platelet GPIIb-IIIa autoantibodies (GTI Pak-Plus, WI, USA) were detectable in the serum.

She underwent 3 immunoadsorption procedures over one week and about five liters of plasma were processed, while she was still on prednisone and mesterolon 50 mg/day (Figure 1a). IgG level decreased from 9.72 g/L to 0.52 g/L at the end of the third procedure, when she received 90 g of IVIgG. Her platelet count rose to 80×10^9/L, but returned to the basal value 13 days later, while PAIgG were still detectable; conversely, anti-platelet GPIIb-IIIa autoantibodies disappeared from the serum. Platelet count then progressively rose to normal in the following 6 months; prednisone and mesterolon were tapered until withdrawal and the patient remains in complete remission so far.

Patient #2. A 71-year old female suffered from severe symptomatic thrombocytopenia (platelets 3×10^9/L) despite treatment with steroids, IVIgG, and vincristine; PAIgG were increased without serum specific anti-platelet autoantibodies while anti-HLA class I antibodies were detectable in the serum. Splenectomy was ruled out because of concurrent personal risk factors. Therapy with danazol was started and the patient underwent EC10 treatment as previously described. About 10 liters of plasma were processed during the immunoadsorption while IgG decreased from 13.1 g/L to 1.29 g/L and anti-HLA antibodies disappeared from the serum. The patient became responsive to platelet concentrates transfused during the first two procedures, and exhibited a transient increase in platelet count (23×10^9/L) when she was...
Programmed versus non-programmed freezing of umbilical cord blood

Programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers. We designed a prospective study to compare programmed and non-programmed freezing for cord blood. Our results suggest the feasibility of non-programmed freezing for umbilical cord blood, simplifying the method and decreasing costs in a cord blood bank.

Sir,

Many authors have established the optimal conditions for cryopreservation of umbilical cord blood to be a controlled cooling rate of 1°C/min. However, programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers. We designed a prospective study to compare programmed and non-programmed freezing for cord blood. For this purpose, 39 cord blood units were collected, volume reduced and cryopreserved in two 25 mL aliquots with 10% DMSO final concentration, following Rubinstein’s method. One of the aliquots was cryopreserved in a controlled rate freezer (Planer Bioimed, Kryo 10) with a cooling-rate of 1°C/min, and the other one was placed directly into a –80°C mechanical freezer (Koxa). After 24 hours, the –80°C frozen cord blood was stored in a liquid nitrogen tank in the vapor phase. After 7 days, the UCB was thawed by submerging the bag in a 37°C water bath and washing the cells with thawing solution containing dextran and human gamma globulin. Blood 1989; 74:2414-7.

Table 1. Recovery of nucleated total cells, CD34+ cells and colony-forming units after thawing.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>TNC x10⁶</td>
<td></td>
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<td>0.067</td>
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<td>40</td>
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<td>CD34 x10⁴</td>
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<td>1.55</td>
<td>1.3</td>
<td>1.02</td>
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<tr>
<td>CFUs x10⁴</td>
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<td>43.85</td>
<td>34.91</td>
<td>34.15</td>
<td>2.1</td>
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<tr>
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<td>33</td>
<td>40.21</td>
<td>27.05</td>
<td>34.55</td>
<td>2.1</td>
<td>131.3</td>
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<tr>
<td>TNC Rec (%)</td>
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<td></td>
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<td>88</td>
<td>89</td>
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<td>61.1</td>
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<tr>
<td>CD34 Rec (%)</td>
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<td>98.8</td>
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<td>CFUs Rec (%)</td>
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<td>70</td>
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<td>11.49</td>
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<td>36.94</td>
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<td>Viability (%)</td>
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<td>71</td>
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<td>69.13</td>
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<td>13.72</td>
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CNT: total nucleated cells. CFUs: colony-forming units. Rec: recovery expressed as percentage.