indicated by \( G \) and counted. IgG fraction (96/1/10) is indicated by culture containing nonadherent human bone marrow mononuclear cells. After (January 10, 1996) or during therapy (October 14, 1997), and added to a soft agar human interleukin-3 was preincubated with the patient's IgG fractions before therapy IL-3 as described previously.8 Glycosylated recombinant human TPO or recombinant in a soft agar medium containing recombinant human TPO or recombinant human interleukin-3 was preincubated with the patient's IgG fractions before therapy however, did not affect human interleukin-3 (IL-3)-induced megakaryocyte colony formation. These data indicate that the IgG autoantibodies can specifically neutralize the in vitro biologic activity of TPO.

The effectiveness of cyA in improving thrombocytopenia strongly suggests an immune-mediated pathogenetic mechanism in the patient. Although it is unclear that anti-TPO autoantibodies is the sole cause of the thrombocytopenia, the recovery of serum TPO levels and peripheral platelet counts appeared to be closely related to the decrease in the antibody levels. Low levels of measurable TPO before cyA therapy could be due to the antibody-mediated increased clearance of TPO or the interference by the antibody in the detection of serum TPO. In either case, the autoantibodies might result in a decrease in the effective TPO concentrations for stimulating megakaryocytopoiesis, leading to thrombocytopenia. In recent clinical trials of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), a very small proportion of healthy volunteers who had received repeated subcutaneous injections of PEG-rHuMGDF-developed antibodies against endogenous TPO and eventually became thrombocytopenic (unpublished data). The present results, together with these observations, suggest that an autoantibody against TPO should be included in the pathogenetic mechanisms underlying AMTP.

**To the editor:**

The preferential expression of CD7 and CD34 in myeloid blast crisis in chronic myeloid leukemia

Chronic myeloid leukemia (CML) is a myeloproliferative disorder that results from the clonal expansion of a pluripotent stem cell, characterized by the Philadelphia chromosome (Ph).1 The clinical course of CML is generally biphasic, representing an initial chronic phase (CP) and a subsequent blast crisis (BC), which is an inevitably terminal event.1 Although almost all the hematopoietic lineages may be involved in this event, two main forms are recognized as lymphoid and myeloid crisis, and herein the latter includes typical myeloid, erythroid, as well as megakaryocytic phenotype. CML is hypothesized to evolve in a multistep fashion that results from the clonal expansion of a pluripotent stem cell, characterized by the Philadelphia chromosome (Ph). The clinical manifestations of CML include chronic phase, accelerated phase and blast crisis. The preferential expression of CD7 and CD34 in myeloid blast crisis in chronic myeloid leukemia

Figure 2. Inhibitory effect of the patient’s IgG on TPO-induced human megakaryocyte colony formation. The megakaryocyte progenitor assay was performed in a soft agar medium containing recombinant human TPO or recombinant human IL-3 as described previously.3 Glycosylated recombinant human TPO or recombinant human interleukin-3 was preincubated with the patient’s IgG fractions before therapy (January 10, 1996) or during therapy (October 14, 1997), and added to a soft agar culture containing nonadherent human bone marrow mononuclear cells. After 14 days of culture, megakaryocyte colonies were immunohistochemically stained and counted. IgG fraction (96/1/10) is indicated by \( [1] \), IgG fraction (97/10/14) is indicated by \( [2] \), and 0.1% bovine serum albumin is indicated by \( [3] \).

**References**

marrow preparations were directly labeled by a mixture of anti-CD34-phycocerythrin (PE) (clone 8G12; Becton Dickinson, San Jose, CA) and anti-CD7-fluorescein isothiocyanate (FITC) (clone 3A1E-12H7; Coulter Inc., Hialeah, FL), or other pairs of antibodies, along with anti-CD45-peridin chlorophyll α protein (PerCP) (Becton Dickinson). After labeling and lysing, nucleated cells were analyzed on FACS Calibur (Becton Dickinson) with an argon-ion laser tuned at 488 nm. On a CD45 versus sideward light scatter (SSC) dot plot, blast cells occupy a unique blast region characterized by intermediate CD45 intensity and low SSC. Cells labeled with mouse IgG2b-FITC and IgG1-PE were used as control to define the nonlabeled population. Cells were defined to be positive when 10% or more blasts were labeled with the specific monoclonal antibodies. We found that CD7 expression was detected in 80.6% of myeloid BC and 67.7% of undifferentiated BC cases but in neither cases of lymphoid BC, all of which unequivocally revealed B-lineage or mixed-lineage phenotypes (Table). On the other hand, CD34 was expressed in 75% of lymphoid and 96.4% of myeloid BC (Table). Analysis of the relationship between CD7 and CD34 expression on blasts of myeloid crisis showed their dual expression in 24 of 27 cases analyzed.

Similar to our results, Urbano-Ispizua et al observed CD7 expression in 7 of 26 cases (27%) of myeloid BC,3 and Martin-Henao et al reported that CD7 was expressed in 4 of 5 cases.1 On the contrary, Nair et al did not point out its expression in myeloid BC.4 There are some variations in the level of CD7 expression among the reports. These variations would reflect the differences in detection methods. Both Urbano-Ispizua et al and Nair et al used indirect immunohistochemical methods, while Martin-Henao et al performed flowcytometry using a conventional gating strategy. In this respect, the method we applied makes it possible to accurately estimate the immunophenotype of the blasts even when they represent the minor population. Our results suggest that myeloid BC coexpressing CD7 and CD34 may represent the maturation arrest of immature myeloid progenitor cells when CD7 is transiently expressed.

To the editor:

Effects of GP IIb/IIIa receptor antagonists on the activated clotting time of heparinized blood

The activated clotting time (ACT) is a functional measure of the intrinsic coagulation pathway that has become a standard monitoring device of heparin. In the Evaluation of c7E3 Fab in the Prevention of Ischemic Complications (EPIC) study, ACT has been measured during percutaneous transluminal coronary angioplasty (PTCA) to minimize thrombotic events and hemorrhagic complications.1,2 To investigate whether a GP IIb/IIIa receptor antagonist influences ACT, Moliterno et al3 reviewed data from the EPIC trial and demonstrated that ACT was more prolonged in heparinized patients treated with c7E3 Fab (abciximab-Reopro) than in those treated with placebo. Subsequently, it was shown in vitro that the addition of c7E3 Fab significantly prolonged ACT of blood anticoagulated with heparin, hirudin, and D-phenylalanine-L- propyl-L-arginyl chloromethyl ketone (PPACK), suggesting that in vivo c7E3 Fab acts not only as an antiplatelet but also as an anticoagulant agent.4 The ACT of in vitro heparinized blood obtained from healthy donors or obtained from patients during cardiopulmonary bypass was prolonged when a platelet inhibitor (prostacyclin or carbacyclin, respectively) was added.5 Since platelet activation leads to generation of thrombin,6 it is not surprising that a coagulation time measured in whole blood might be dependent on platelet function. Consequently, the presence of platelet inhibitors can influence ACT.

In current practice, 2 automated devices, HemoTec (Medtronic Inc, Parker, CO) and Hemochron (ITC Technidyne Corp, Edison, NJ) are commonly used for measuring ACT, each one using a different technique to determine clot formation in the presence of diatomaceous earth or kaolin as contact activator.7 As shown in patients undergoing coronary angioplasty,8 Hemochron and Hemochron ACT measurements cannot be used interchangeably after heparin administration because Hemochron values are about 28% higher than the HemoTec values.

Here we report that when ACT is determined by the Hemochron device (Figure 1A, B), the time elapsed between blood sampling in

<table>
<thead>
<tr>
<th>Type of crisis</th>
<th>No of cases</th>
<th>CD7</th>
<th>CD34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid</td>
<td>39</td>
<td>29/36 (80.6)</td>
<td>27/28 (96.4)</td>
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<tr>
<td>Lymphoid</td>
<td>10</td>
<td>0/9 (0)</td>
<td>3/4 (75.0)</td>
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<tr>
<td>Undifferentiated</td>
<td>3</td>
<td>2/3 (67.7)</td>
<td>0/0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>31/48 (64.6)</td>
<td>30/32 (93.8)</td>
</tr>
</tbody>
</table>

Positive numbers per analyzed sample numbers are indicated. Percentages are shown in parentheses.

References

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Nobuharu Kosugi, Arinobu Tojo, Hitoshi Shinzaki, Tokiko Nagamura-Inoue and Shigetaka Asano