To the editor:

Autoantibodies neutralizing thrombopoietin in a patient with amegakaryocytic thrombocytopenic purpura

Amegakaryocytic thrombocytopenic purpura (AMTP), first reported by Korn,1 is a rare disease characterized by severe thrombocytopenia associated with a total absence or a selective decrease in bone marrow megakaryocytes. Previous studies suggest a variety of pathogenetic mechanisms for AMTP, such as the intrinsic defect of platelet production, a decreased availability or function of TPO might lead to a form of AMTP.2 As shown in Figure 1, the patient’s serum before therapy had the highest level of anti-TPO IgG antibodies during the scope of the study. With cyA therapy, the antibody levels declined and did not return to the pretreatment value until the end of the study. Endogenous TPO levels appear to be regulated by c-Mpl, the receptor for TPO, expressed on platelets and megakaryocytes, a mechanism of end-cell regulation. Previous studies showed that serum TPO levels were markedly elevated in patients with AMTP before therapy.6 In contrast to these findings, Figure 1 shows that in spite of severe thrombocytopenia, the pretreatment level of TPO (0.21 fmol/mL) was lower than baseline values of normal subjects (0.33 to 1.72 fmol/mL) as previously reported.7 Throughout the study period, circulating platelet counts appeared to increase in response to the elevation in endogenous TPO levels (Figure 1). In an effort to characterize the patient’s anti-TPO antibody, we examined the effect of the IgG fractions from the patient’s serum on the growth of megakaryocyte colonies from adherent cell-depleted normal human bone marrow mononuclear cells (Figure 2). The IgG fraction before cyA therapy or during therapy (October 14, 1997) completely or partially reduced the number of megakaryocyte colonies stimulated with 0.2 ng/mL of glycosylated recombinant human TPO, respectively. This inhibition of megakaryocyte colony formation was able to be overcome by increasing the concentration of human TPO in the cultures to 10 ng/mL. The IgG fractions, 8. Vanham G, Penne L, Devalck J, et al. Decreased CD40 ligand induction in CD4 T cells and dysregulated IL-12 production during HIV infection. Clin Exp Immunol. 1999;117:335-342.

Figure 1. Changes in anti-TPO antibody levels, TPO concentrations, and platelet counts during cyA treatment. Anti-TPO IgG antibodies were measured with a solid-phase radioimmunoassay. Briefly, microtitre plates were incubated with 4 µg/mL of recombinant human TPO at 4°C overnight and washed. Test sera diluted at 1:10 in phosphate-buffered saline were added to the wells and incubated at 37°C for 1 hour. After washing, the wells were incubated with radiiodinated protein A at room temperature for 2 hours. Finally, the radioactivity bound to the wells was measured with a gamma counter. Data from the assay validation using rabbit polyclonal IgG antibodies against human TPO showed that the intra- and interassay coefficients of variation ranged from 6.3% to 10.3% and from 7.9% to 13.4%, respectively. Serum TPO levels were measured as described previously.
indicated by and counted. IgG fraction (96/1/10) is indicated by

Figure 2. Inhibitory effect of the patient’s IgG on TPO-induced human mega-
karyocyte 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. 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 □. IgG fraction (97/10/14) is
indicated by △, and 0.1% bovine serum albumin is indicated by ■.

however, did not affect human interleukin-3 (IL-3)-induced mega-
karyocyte colony formation. These data indicate that the IgG autoanti-
bodies 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 autoantibody 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 megakaryocypopoiesis, leading to thrombocytopenia.
In recent clinical trials of pegylated recombinant human megakaryo-
cyte 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 obser-
vations, suggest that an autoantibody against TPO should be
included in the pathogenetic mechanisms underlying AMTP.

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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). The clinical
course of CML is generally biphasic, representing an initial chronic
phase (CP) and a subsequent blast crisis (BC), which is an
invariably terminal event. 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
from the preleukemialike CP into the most malignant BC. From
this viewpoint, a certain fraction of Ph-positive stem/progenitor
cells in CP might acquire additional genetic abnormalities and
represent maturation arrest at a given stage of differentiation
program. Then, the immunophenotype of blast cells in BC might
reflect the antigenic profile of such a fraction of stem/progeni-
tor cells in CP. In this sense, some notice should be given to
the 2 recent papers in which it was described that the popula-
tion of CD34+CD7+ cells are higher in CML-CP than in normal
donors.

We retrospectively examined the immunophenotype of the blast
population in 52 consecutive, well-characterized cases of CML-BC
diagnosed in our institute, using multicolor flow cytometric
analysis. According to the morphological, conventional cytochemi-
cal reactions and the lineage-specific immunological markers such
as CD3, CD10, CD13, CD19, CD33, and CD41a, these cases were
classified as follows: lymphoid (n = 10; 19.2%), myeloid (n = 39;
75.0%), and undifferentiated (n = 3; 5.7%). Myeloid crisis con-
tains myelomonocytic (n = 1) and megakaryocytic (n = 6) crisis
as defined by the expression of CD41a. Flow cytometric analysis
was performed in a significant part of our cases according to
the method as previously described. In brief, diluted bone

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