ORIGINAL ARTICLE

Paraneoplastic Thrombocytosis in Ovarian Cancer

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ABSTRACT

BACKGROUND

The mechanisms of paraneoplastic thrombocytosis in ovarian cancer and the role that platelets play in abetting cancer growth are unclear.

METHODS

We analyzed clinical data on 619 patients with epithelial ovarian cancer to test associations between platelet counts and disease outcome. Human samples and mouse models of epithelial ovarian cancer were used to explore the underlying mechanisms of paraneoplastic thrombocytosis. The effects of platelets on tumor growth and angiogenesis were ascertained.

RESULTS

Thrombocytosis was significantly associated with advanced disease and shortened survival. Plasma levels of thrombopoietin and interleukin-6 were significantly elevated in patients who had thrombocytosis as compared with those who did not. In mouse models, increased hepatic thrombopoietin synthesis in response to tumor-derived interleukin-6 was an underlying mechanism of paraneoplastic thrombocytosis. Tumor-derived interleukin-6 and hepatic thrombopoietin were also linked to thrombocytosis in patients. Silencing thrombopoietin and interleukin-6 abrogated thrombocytosis in tumor-bearing mice. Anti–interleukin-6 antibody treatment significantly reduced platelet counts in tumor-bearing mice and in patients with epithelial ovarian cancer. In addition, neutralizing interleukin-6 significantly enhanced the therapeutic efficacy of paclitaxel in mouse models of epithelial ovarian cancer. The use of an antiplatelet antibody to halve platelet counts in tumor-bearing mice significantly reduced tumor growth and angiogenesis.

CONCLUSIONS

These findings support the existence of a paracrine circuit wherein increased production of thrombopoietic cytokines in tumor and host tissue leads to paraneoplastic thrombocytosis, which fuels tumor growth. We speculate that countering paraneoplastic thrombocytosis either directly or indirectly by targeting these cytokines may have therapeutic potential. (Funded by the National Cancer Institute and others.)

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This article (10.1056/NEJMoa1110352) was updated on October 18, 2012, at NEJM.org.

N Engl J Med 2012;366:610-8.

Platelets are highly reactive cellular effectors of hemostasis, immunity, and inflammation.[1] The concept that platelets play key roles in cancer growth and metastasis is long-standing. In fact, the clinical observation that thrombocytosis (defined as a platelet count of >450,000 per cubic millimeter) occurs in patients with solid tumors was made more than 100 years ago.[2,3] Nearly 40% of persons incidentally found to have platelet counts exceeding 400,000 per cubic millimeter in the absence of iron deficiency and benign inflammatory conditions have an occult cancer, most commonly a primary gastrointestinal, lung, breast, or ovarian cancer.[4] Beyond clinical observations, experimental evidence suggests that platelets actively promote cancer progression through diverse mechanisms, including protection of cancer cells from immune surveillance, negotiation of cancer-cell arrest in the microvasculature, and stimulation of angiogenesis.[5]

Given the short life span of a circulating platelet, approximately 10^{11} platelets must be produced daily to maintain a normal platelet count in adults.[6,7] This high baseline level of production has the potential to markedly increase in the context of cancer. The mechanisms underlying this surge in platelets as well as its biologic significance are not well understood and are the focus of the current study. We investigated the potential clinical and biologic implications of a paracrine circuit that promotes megakaryopoiesis and thrombocytosis in the context of ovarian cancer.

Methods

Clinical Data

After approval by the local institutional review boards, clinical data, including history of coexisting inflammatory conditions, were collected on 619 consecutive patients with primary epithelial ovarian cancer from four U.S. academic medical centers. The effect of treatment with an antihuman interleukin-6 antibody (siltuximab [CNTO 328], at a dose of 5.4 mg per kilogram of ideal body weight infused once every 2 weeks) on platelet counts in 18 patients with ovarian cancer was evaluated as part of a phase 1 and 2 study (EudraCT number, 2006-005704-13).[8] Patients provided written informed consent for participation in this trial and for use of their banked specimens in the laboratory studies pertaining to this investigation.

Mouse Models

All in vivo experiments in animals were approved by the institutional animal care and use committee. The development and characterization of the orthotopic mouse models of epithelial ovarian cancer have been described previously.[9-13] Additional details regarding in vivo experiments with small interfering RNA (siRNA) delivery (Fig. 1A and 1B in the Supplementary Appendix, available with the full text of this article at NEJM.org), siltuximab, antiplatelet antibody (Emfret Analytics) (Fig. 2A through 2E in the Supplementary Appendix), inflammatory agents, and platelets from transgenic mice that express yellow fluorescent protein (YFP) are described in the Supplementary Appendix.

Laboratory Measures

Plasma levels of key thrombopoietic factors were measured with the use of human cytokine immunobead panels (Milliplex, Millipore) coupled with a multiplex assay (involving xMAP technology, Luminex) or with enzyme-linked immunosorbent assays (ELISA) (Quantikine, R&D Systems). Real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays were performed with whole RNA extracted from tumor specimens (obtained from 46 patients) or liver specimens (from 10 patients and 10 mice) with the use of primer sets specific for human interleukin-6 and murine or human thrombopoietin, respectively, as previously described.[9,14,15] Proliferation index and microvessel density were evaluated by staining for Ki67 and CD31 antigens, respectively.[16,17] Dual immunofluorescence staining for CD31 and desmin and for CD31 and terminal deoxynucleotidyl transferase dUTP biotin nick end labeling (TUNEL) was used to assess pericyte coverage and apoptotic tumor and endothelial cells, respectively.[18] The in vitro effects of gel-filtered platelets (see the Supplementary Appendix) on cell migration and proliferation in epithelial ovarian cancer were evaluated with the use of two-chamber chemotaxis and bromodeoxyuridine flow-cytometric assays (Click-iT EdU, Invitrogen), respectively.[19]

Statistical Analysis

Associations between platelet count and clinicopathological variables were tested in 619 patients with newly diagnosed epithelial ovarian cancer with the use of Fisher's exact test. Kaplan–Meier survival curves were generated and compared with the use of a log-rank statistic. Multivariate analysis

of these data was performed with the Cox proportional-hazards model. To determine whether plasma thrombopoietic cytokine levels were associated with platelet counts in 150 patients with newly diagnosed epithelial ovarian cancer, we calculated Spearman's rank-correlation coefficient for each pairwise association and tested for a significant difference from 0. We used Wilcoxon rank-sum tests to determine differences in thrombopoietic cytokines between patients with normal platelet counts (150,000 to 450,000 per cubic millimeter) and those with high counts (>450,000 per cubic millimeter).

For interleukin-6 analysis, we used a level close to the median to classify patients with newly diagnosed epithelial ovarian cancer as having low interleukin-6 levels (≤10 pg per milliliter, 143 patients) or high levels (>10 pg per milliliter, 167 patients). Pearson's correlation was used to calculate correlations between continuous variables. For preclinical data, Student's t-test was used to test differences in sample means, with a sample of 10 mice per group (80% power to detect a 50% reduction in tumor weight at a 5% level of statistical significance). All testing was two-sided, and a P value of less than 0.05 was considered to indicate statistical significance. All analyses were completed with SAS software, version 9.1 for PC (SAS Institute).

RESULTS

CLINICAL IMPLICATIONS OF PARANEOPLASTIC THROMBOCYTOSIS

The mean (±SD) platelet count in patients with epithelial ovarian cancer and thrombocytosis was 558,410±90,010 per cubic millimeter (median, 542,000 per cubic millimeter) and without thrombocytosis was 316,570±70,140 per cubic millimeter (median, 318,000 per cubic millimeter) (Table 1 in the Supplementary Appendix). Thirty-one percent of the patients (192 of 619) had thrombocytosis at the time of initial diagnosis of epithelial ovarian cancer. Less than 2% of the patients had a coexisting nonmalignant inflammatory condition, and none of these patients had thrombocytosis at initial presentation. Although women with thrombocytosis had slightly lower hemoglobin levels than those with normal platelet counts (mean hemoglobin level, 11.9 vs. 12.7 g per deciliter; P<0.001), mean corpuscular volume and serum ferritin levels did not differ significantly between the two groups (Table 2 in the Supplementary Appendix). These data suggest that other inflammatory conditions and iron deficiency are unlikely to be responsible for the thrombocytosis observed in women with epithelial ovarian cancer.

Patients with thrombocytosis were significantly more likely to have advanced-stage disease, vascular thromboembolic complications, and higher preoperative levels of cancer antigen 125 than those with normal platelet counts (Fig. 3 in the Supplementary Appendix). Women with platelet counts of more than 450,000 per cubic millimeter had a significantly shorter median time to disease progression than those with normal platelet counts (Fig. 1A). The median overall survival among patients with thrombocytosis was 2.62 years, as compared with 4.65 years among those with normal platelet counts (P<0.001) (Fig. 1B). In the multivariate model that included age, disease stage, tumor grade, histologic type, and extent of surgical cytoreduction, thrombocytosis remained an independent predictor of compromised survival (P<0.001) (Table 3 in the Supplementary Appendix).

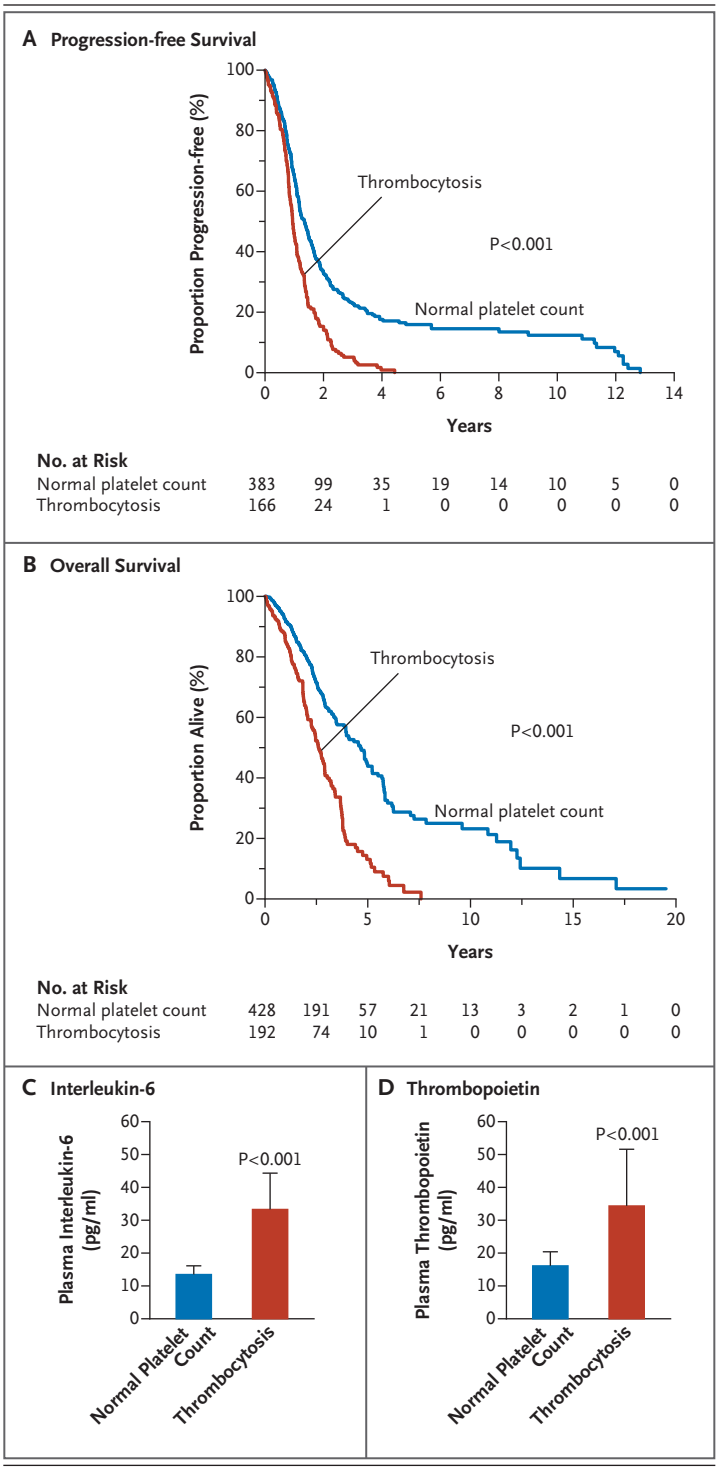
PARANEOPLASTIC THROMBOCYTOSIS IN MOUSE MODELS OF EPITHELIAL OVARIAN CANCER

On the basis of the clinical findings described above, we evaluated the effect of cancer on platelet counts in orthotopic mouse models of epithelial ovarian cancer. As compared with platelet counts in control mice, those in mice with epithelial ovarian cancer were increased by 30 to 130% (P=0.009 for HeyA8, P=0.003 for A2780ip2, and P=0.001 for 2774 mouse models) (Fig. 4A in the Supplementary Appendix). Platelet counts correlated significantly with tumor burden (r=0.78, P<0.001) and number of intraperitoneal metastases (r=0.65, P=0.004) (Fig. 4B and 4C in the Supplementary Appendix). Platelet counts were also increased by 59 to 64% in two syngeneic mouse models of ovarian cancer (P=0.01) (Fig. 4D in the Supplementary Appendix). To assess generalizability, we also quantified platelets in mouse models of breast cancer (GILM2 cells), uterine cancer (Ishikawa cells), and pancreatic cancer (heterotopic human pancreatic xenografts). Platelet counts were significantly increased in the models of uterine and pancreatic cancer but not in the breast-cancer model (Fig. 4E in the Supplementary Appendix).

To better understand the temporal relationship between tumor growth and thrombocytosis, we carried out a longitudinal assessment of platelet counts in HeyA8–luciferase-positive tumor-bearing

Figure 1. Clinical Significance of and Thrombopoietic Cytokines Associated with Paraneoplastic Thrombocytosis in Patients with Epithelial Ovarian Cancer.

Panel A shows Kaplan–Meier survival curves for progression-free survival according to platelet count at initial diagnosis among patients with epithelial ovarian cancer, and Panel B shows curves for overall survival. Panel C shows mean plasma levels of interleukin-6 in 104 patients with epithelial ovarian cancer and normal platelet counts and 46 patients with epithelial ovarian cancer and thrombocytosis, and Panel D shows mean plasma levels of thrombopoietin in the same patients. T bars indicate standard deviations. Thrombocytosis was defined as a platelet count of more than 450,000 per cubic millimeter.



mice. Platelet counts began to rise when a tumor was detected by bioluminescence imaging and increased in parallel with grossly measurable disease (Fig. 5 in the Supplementary Appendix).

AN UNDERLYING MECHANISM OF PARANEOPLASTIC THROMBOCYTOSIS

To test the hypothesis that factors produced by cancer cells or host tissues (or both) stimulate megakaryopoiesis and paraneoplastic thrombocytosis, we first enumerated megakaryocytes in the bone marrow and spleens from tumor-bearing mice. Mean platelet counts strongly correlated with mean medullary and splenic megakaryocyte counts ($r=0.95$, $P=0.01$). As compared with medullary and splenic megakaryocyte counts in controls, those in tumor-bearing mice were increased by a factor of 2 to 3 and 7 to 13, respectively ($P<0.001$) (Fig. 6A in the Supplementary Appendix). To determine whether the findings would be similar in patients with epithelial ovarian cancer, we surveyed our clinical data set for women who underwent a bone marrow biopsy. Among the eight patients in whom a bone marrow biopsy was performed, platelet counts correlated with megakaryocyte density (two patients with thrombocytosis had megakaryocyte hyperplasia, four patients had normal platelet and megakaryocyte counts, and two patients had thrombocytopenia and megakaryocyte hypoplasia) (Fig. 6B in the Supplementary Appendix).

Next, we considered the possible involvement of humoral factors known to physiologically regulate megakaryopoiesis and platelet production. We quantified plasma levels of 10 key thrombopoietic factors in an independent cohort of 150 patients with newly diagnosed epithelial ovarian cancer (31% of whom had thrombocytosis, a frequency consistent with that in the initial cohort). Platelet counts correlated significantly with plasma interleukin-6 levels (Spearman's rank-correlation coefficient, 0.37; $P<0.001$) and thrombopoietin levels (Spearman's rank-correlation coefficient, 0.29; $P<0.001$) (Table 4 in the Supplementary Appendix). Plasma levels of interleukin-6 and throm-

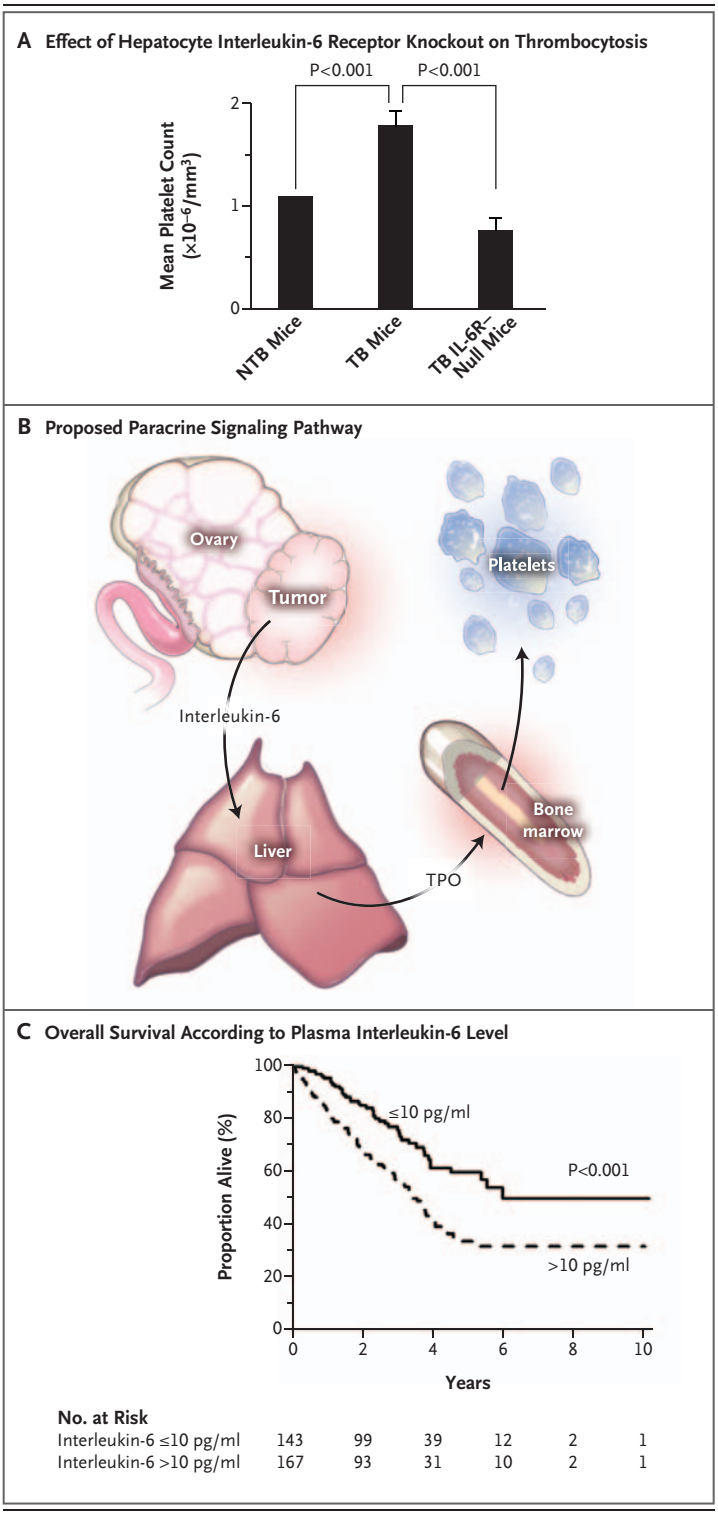


Figure 2. Underlying Mechanism of Paraneoplastic Thrombocytosis in Epithelial Ovarian Cancer.

Panel A shows mean platelet counts in non–tumor-bearing (NTB) mice, tumor-bearing (TB) mice, and TB mice that lacked functional hepatocyte interleukin-6 receptors (TB IL-6R–null mice). T bars indicate standard errors; for NTB mice, the standard error is too small to be shown. Panel B shows the proposed paracrine signaling pathway that mediates paraneoplastic thrombocytosis in epithelial ovarian cancer. Interleukin-6 secreted by ovarian cancer cells stimulates hepatic thrombopoietin (TPO) production. This drives thrombopoiesis in the bone marrow, giving rise to thrombocytosis. Panel C shows Kaplan–Meier survival curves for overall survival according to plasma interleukin-6 levels. Median overall survival among patients with plasma interleukin-6 levels of more than 10 pg per milliliter was 3.38 years, as compared with 5.99 years among those with levels of 10 pg per milliliter or less.

bopoietin were significantly elevated in patients with thrombocytosis (Fig. 1C and 1D, and Table 5 in the Supplementary Appendix).

Plasma levels of human interleukin-6, but not mouse interleukin-6, were significantly elevated in all mouse models of epithelial ovarian cancer that were tested (Fig. 7A in the Supplementary Appendix). Thrombopoietin is mainly synthesized by the liver at a fixed rate, but little is known about the influence of disease states such as cancer on hepatic thrombopoietin production.[20] In liver specimens resected from both control and tumor-bearing mice, levels of hepatic thrombopoietin messenger RNA were 38 to 64% higher in tumor-bearing mice ($P=0.43$ for the 2774 mouse model and $P<0.001$ for the A2780ip2 and HeyA8 mouse models of epithelial ovarian cancer) (Fig. 7B in the Supplementary Appendix).

On the basis of data suggesting that interleukin-6 enhances hepatic thrombopoietin synthesis,[21,22] we measured platelet counts and plasma thrombopoietin levels in tumor-bearing ($ID8^{VEGF-164}$ syngeneic model) mice that lacked functional hepatocyte interleukin-6 receptors. Thrombocytosis was not observed in these mice (Fig. 2A). Furthermore, the mean plasma thrombopoietin level was 33% lower in the tumor-bearing mice that lacked functional hepatocyte interleukin-6 receptors than in tumor-bearing mice with functional receptors (flox/flox controls) (Fig. 7C in the Supplementary Appendix). These findings suggest that increased hepatic thrombopoietin synthesis in response to tumor-derived interleukin-6 is an underlying mechanism of paraneoplastic thrombocytosis (Fig. 2B).

To determine whether this paracrine signaling is the basis for paraneoplastic thrombocytosis in humans, we quantified tumor and plasma interleukin-6 levels in a subset of 46 patients with newly diagnosed epithelial ovarian cancer, using

a quantitative RT-PCR assay and ELISA, respectively. A strong correlation between tumor and plasma interleukin-6 levels was observed ($r=0.8$, $P<0.001$) (Fig. 8A in the Supplementary Appendix). Moreover, mean tumor interleukin-6 levels were significantly higher in patients with thrombocytosis than in those with normal platelet counts (Fig. 8B in the Supplementary Appendix). Interleukin-6 levels were also significantly associated with plasma thrombopoietin levels (Spearman's rank-correlation coefficient, 0.6; $P<0.001$). We also used a quantitative RT-PCR assay to examine 10 liver samples removed from patients with epithelial ovarian cancer during cytoreductive surgery. Hepatic thrombopoietin expression closely paralleled platelet counts ($r=0.64$, $P=0.04$) (Fig. 8C in the Supplementary Appendix).

To better understand the clinical significance of this paracrine-mediated paraneoplastic thrombocytosis, we evaluated the effect of circulating interleukin-6 levels on progression-free and overall survival among 310 patients with epithelial ovarian cancer. In univariate analyses, patients with plasma interleukin-6 levels that were 10 pg per milliliter or lower had improved progression-free survival (Fig. 8D in the Supplementary Appendix) and overall survival (Fig. 2C), as compared with patients who had plasma interleukin-6 levels that were higher than 10 pg per milliliter. In multivariate models, plasma interleukin-6 levels of more than 10 pg per milliliter and platelet counts of more than 450,000 per cubic millimeter remained independent predictors of a poor prognosis even after adjustment for stage, grade, and volume of residual disease after cytoreductive surgery.

Next, to ascertain whether interleukin-6 and thrombopoietin play functional roles in paraneoplastic thrombocytosis, we tested the effect of silencing these factors on thrombocytosis in the A2780ip2 orthotopic mouse model. The combination of interleukin-6 and thrombopoietin siRNA was most effective, completely abrogating thrombocytosis (Fig. 3A). We also explored the therapeutic value of targeting interleukin-6 with siltuximab (humanized anti–interleukin-6 antibody), paclitaxel (a chemotherapeutic agent commonly used in epithelial ovarian cancer), or the two agents combined. Siltuximab or paclitaxel monotherapy significantly reduced tumor growth in mouse models of epithelial ovarian cancer (2774 and A2780ip2 models). Combination treatment was most effective, reducing tumor growth by more than 90% in both models. Assessment of platelet counts at the conclusion of treatment revealed that siltuximab blocked the thrombocytosis characteristic of untreated tumor-bearing mice (Fig. 9A and 9B in the Supplementary Appendix). Moreover, platelet counts rebounded by 34% within 10 days after the end of siltuximab therapy, pointing to the direct role of tumor-derived interleukin-6 in thrombocytosis.

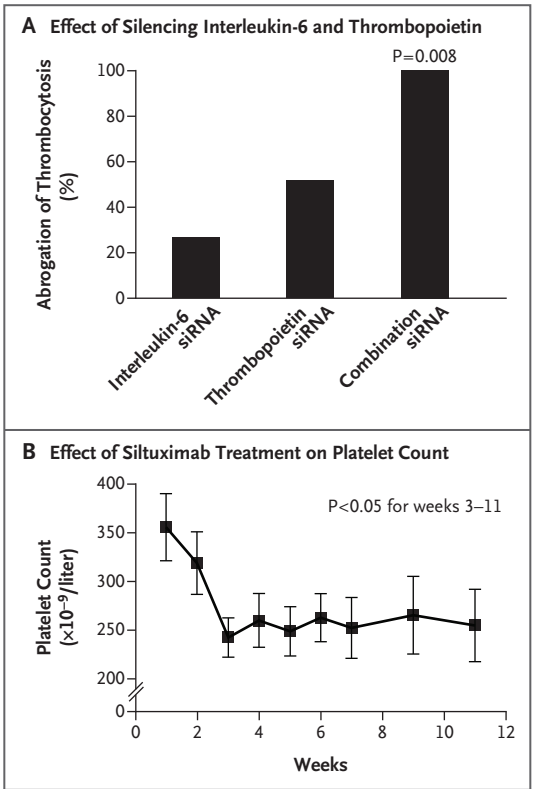


Figure 3. Functional Role of Interleukin-6 and Thrombopoietin in Paraneoplastic Thrombocytosis.

Panel A shows the effect of silencing interleukin-6 alone, thrombopoietin alone, and the two in combination on paraneoplastic thrombocytosis in mice with epithelial ovarian cancer. The P value is for the difference in platelet counts between combination and control siRNA treatment. Panel B shows the effect of siltuximab treatment (every 2 weeks) on platelet counts in patients with epithelial ovarian cancer. I bars indicate standard deviations. The term siRNA denotes small interfering RNA.

To support these in vivo data with human clinical data, we tracked platelet counts in a cohort of patients with ovarian cancer who received treatment with single-agent siltuximab every 2 weeks for 12 weeks. Anti–interleukin-6 treatment resulted in a significant and sustained reduction in platelet counts, from a mean count of $356\times10^9\pm147\times10^9$

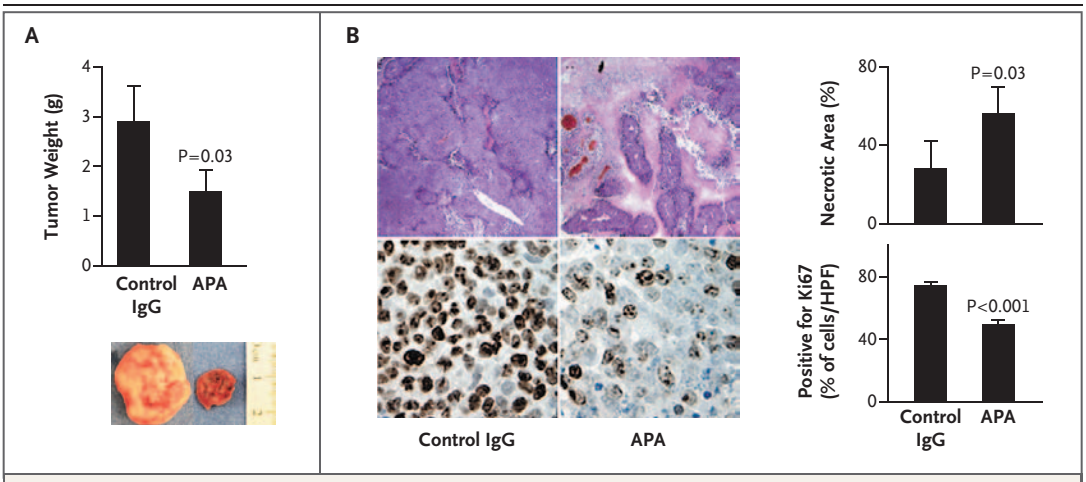


Figure 4. Antitumor Effects of Blocking Paraneoplastic Thrombocytosis.

Panel A shows the mean tumor weight in mice after treatment with either antiplatelet antibody (APA) or control IgG. Representative macroscopic appearances of tumors at necropsy are shown below the histogram. Panel B shows the effect of APA versus control IgG on tumor-cell viability (upper photomicrographs) and proliferation (lower photomicrographs). The corresponding histograms show the mean necrotic tumor area and the number of cells that were positive for Ki67, normalized to the total cell count per high-power field (HPF). T bars indicate standard errors.

per liter before treatment to $243\times10^9\pm85\times10^9$ per liter after 3 weeks of treatment (P=0.009) (Fig. 3B).

BIOLOGIC SIGNIFICANCE OF PARANEOPLASTIC THROMBOCYTOSIS

To evaluate the biologic role of platelets in tumor growth, we used an antiplatelet antibody directed against mouse glycoprotein 1b α (at a dose of 0.5 μg per gram of body weight) to induce a sustained 50% reduction in platelets in the A2780ip2 mouse model. Halving the circulating platelet count inhibited tumor growth by 50% (P=0.03) (Fig. 4A). Microscopical examination of tumors from mice treated with antiplatelet antibody revealed substantial coagulative necrosis (Fig. 4B). As compared with the use of control IgG, the use of antiplatelet antibody to deplete platelets resulted in a 44% decrease in proliferation (P<0.001) (Fig. 4B) and a 51% reduction in microvessel density (P<0.001) (Fig. 10A in the Supplementary Appendix). Platelet depletion reduced pericyte coverage by 60% (P<0.001); tumor microvessels were predominantly devoid of pericytes, and existing pericytes appeared to be loosely attached (Fig. 10B in the Supplementary Appendix).[18,23] Platelet depletion with the use of antiplatelet antibody, as compared with control IgG, resulted in an increase in tumor-cell and endothelial-cell apoptosis by a factor of four and a factor of eight, respectively (P=0.008 and P<0.001, respectively) (Fig. 10C in the Supplementary Appendix).

We next explored whether platelets extravasate into the tumor bed, ascites, or both, sites where direct interactions with cancer cells are more opportune. Platelets from transgenic mice that express YFP under the control of the promoter of platelet factor 4 were transfused into tumor-bearing recipient mice (Fig. 5A). YFP-labeled platelets were detected in the tumor bed, primarily in a perivascular location, and were found to be free-floating and adherent to tumor cells in ascites (Fig. 5B).

To determine the extent to which these observations may be tumor-specific, we compared the number of native, extravascular platelets in tumor samples, in the peritoneum after the induction of chemical peritonitis, and in subcutaneous tissues after the induction of a foreign-body reaction. Extravasated platelets were not observed in the context of a foreign-body inflammatory reaction and were rarely observed in the peritoneum after a pro-inflammatory stimulus. However, abundant platelets were detected in the tumor microenvironment apart from the vasculature (Fig. 5C). On the basis of finding platelets in contact with tumor cells outside the bloodstream, we assessed the functional effects of plasma-purified platelets on the proliferation and migration of epithelial ovarian cancer cells. Platelets significantly stimulated cell proliferation (Fig. 11A in the Supplementary Appendix) and migration (P<0.001 for the three cell lines) (Fig. 11B in the Supplementary Appendix).

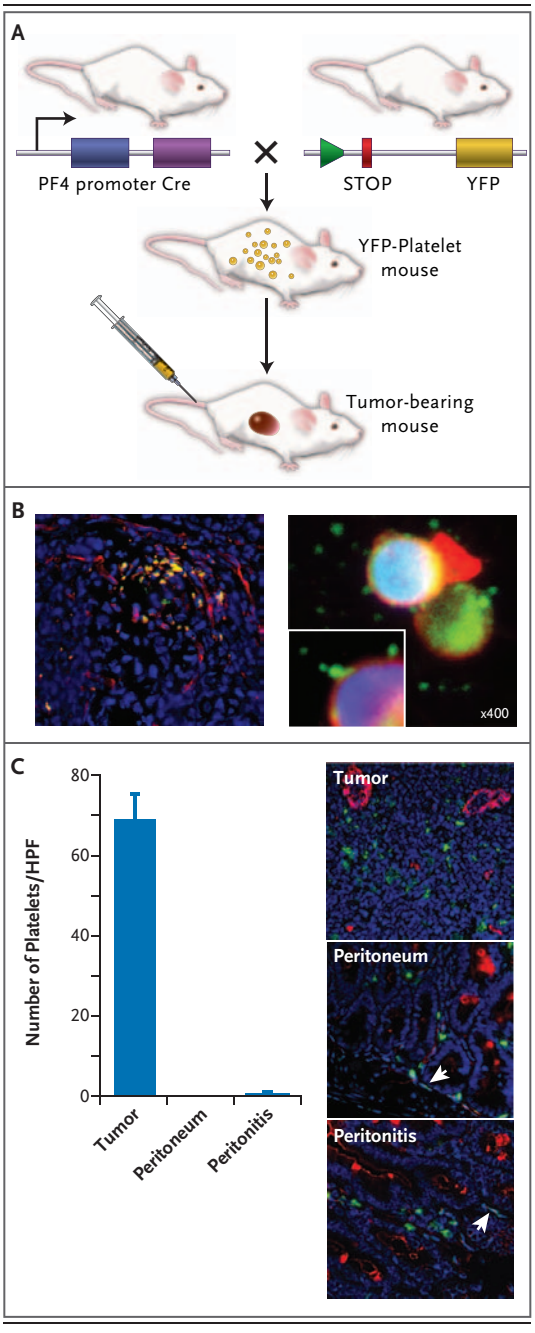


Figure 5. Platelets in the Tumor Microenvironment.

Panel A shows the method used for detecting extravasated platelets. Platelets isolated from female transgenic C57BL/6 mice that express yellow fluorescent protein (YFP) under the control of the promoter of platelet factor 4 (PF4) were transfused by tail-vein injections into tumor-bearing mice. After 1 hour, ascites was removed by paracentesis, and intravital fixation was performed before the resection of tumor specimens. Panel B shows extravascular YFP platelets in solid tumor (left: CD31, red; nuclei, blue) and in ascites (right: tumor cells, red). Panel C shows representative photomicrographs of tumor, normal peritoneum, and peritoneum after the induction of peritonitis, with immunofluorescence staining for CD31 and CD42b antigens to label the vasculature (red) and endogenous platelets (green), respectively. The arrowheads indicate colocalization of CD31 and CD42b, indicative of intravascular platelets. The bar graph depicts the number of extravascular platelets per five random high-power fields (HPF) for each condition. T bars indicate standard errors.

DISCUSSION

Our study shows that increased thrombopoietic cytokine production by tumor and host tissues is a major driving force for paraneoplastic thrombocytosis. In particular, this process is mediated by hepatic thrombopoietin synthesis that is increased in response to excessive tumor-derived interleukin-6, thereby increasing platelet counts, which in turn promote tumor growth, creating a feed-forward loop.

Although paraneoplastic thrombocytosis is a well-recognized phenomenon in patients with cancer, the mechanisms underlying this observation are not fully defined. Findings from this study, derived from converging clinical information as well as pharmacologic and genetic manipulations, not only reveal the complex paracrine signaling responsible for paraneoplastic thrombocytosis in ovarian cancer but also point to the pivotal role of platelets in driving the biologic mechanisms of malignant tumors. For example, the secretory activities of platelets may stabilize the tumor vasculature and prevent intratumoral hemorrhage. The platelet-derived factors involved in maintaining angiogenic microvessel integrity and the cell type that platelets influence most are not fully known.[24] Given the role of pericytes in supporting and stabilizing small blood vessels, it is possible that platelets regulate tumor-vasculature homeostasis, in part by nurturing pericytes. Prospective collection and in-depth analysis of platelets from patients with cancer will be required to fully understand the cancer-promoting role of platelets.

This aberrant paracrine signaling may have important clinical ramifications, since paraneoplastic thrombocytosis arises in a substantial proportion of patients with cancer. For example, one in three women who have received a diagnosis of ovarian cancer have thrombocytosis and are thus at substantially increased risk for advanced disease, vascular thromboembolic complications, and

compromised disease-specific survival. Given that paraneoplastic thrombocytosis portends adverse clinical outcomes, countering it and using direct antiplatelet strategies have the potential to serve as a valuable therapeutic approach in humans.

Although the present investigation does not test this strategy in patients with cancer, such a premise is directly supported by epidemiologic studies of drugs known to interfere with cross-talk between platelets and tumor cells. Several prospective clinical trials have shown that low-molecular-weight heparin improves survival among patients with cancer, independently of the prevention of vascular thromboembolic complications due to anticoagulation.[25] In addition, a recent large prospective cohort study revealed that daily use of aspirin after the diagnosis of colorectal cancer decreases cancer-specific and overall mortality.[26,27] These data warrant further consideration, particularly given that paraneoplastic thrombocytosis may be not simply an epiphenomenon of cancer progression but a contributor to the process.

Supported in part by a Gail MacNeil KOH Research Award from the Gynecologic Cancer Foundation; grants (CA109298, P50 CA083639, P50 CA098258, CA128797, RC2GM092599, CA140933, CA016672, U54 CA151668, and R01CA104825) from the National Institutes of Health; a Program Project Development Grant from the Ovarian Cancer Research Fund; grants (OC073399, W81X-WH-10-1-0158, and BC085265) from the Department of Defense; the Baylor College of Medicine and M.D. Anderson Cancer Center Multidisciplinary Research Program; the Zarrow Foundation; the Marcus Foundation; the Estate of C.G. Johnson, Jr.; the United Kingdom Medical Research Council; the Blanton–Davis Ovarian Cancer Research Program; the Laura and John Arnold Foundation; the RGK Foundation; the Bettyann Asche Murray Distinguished Professorship; and training grants (T32 CA101642 supported Drs. Stone, Nick, Bottsford-Miller, and King and T32 CA009666 supported Dr. Pecot) from the National Cancer Institute. The siltuximab trial was funded by the United Kingdom Medical Research Council and sponsored and monitored by Queen Mary, University of London.

Dr. Stone reports being listed as one of the inventors on a U.S. patent entitled "Use of Selective Adenosine A1 Receptor Allosteric Enhancers to Manipulate Angiogenesis." No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Donna Reynolds, Robert Langley, Ph.D. (Department of Cancer Biology, M.D. Anderson Cancer Center), De-Yu Shen, and Nicholas B. Jennings for their technical assistance and helpful discussions, as well as Bueso-Ramos, M.D., Ph.D. (Department of Hematopathology, M.D. Anderson Cancer Center), for his hematopathology expertise.

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