

## EDITORIAL

# Thrombopoietin

By Shirley Ebbe

**I**T HAS BEEN CLEAR for almost 20 years that platelet production is subject to homeostatic regulatory mechanisms that result in stimulation when the platelet count is low<sup>1</sup> and suppression when it is high.<sup>2</sup> In spite of elapsed time, the physiologic processes that are involved in those regulatory mechanisms are not clear. Thrombocytopoiesis may be regulated by a humoral thrombopoietin, much as erythropoiesis is regulated by erythropoietin. This is an attractive hypothesis which seems to be gaining in popularity and for which there is some experimental support. The analogy implies that a low platelet concentration would cause a specific hormone, thrombopoietin, to be produced, that the hormone would stimulate megakaryocytopoiesis, and that production of the hormone would be suppressed by thrombocytosis.

A number of investigators have found that the passive transfer of plasma or serum from thrombocytopenic animals or human beings to assay animals will result in increased production of platelets in the recipients. Two reports<sup>3,4</sup> indicate that thrombopoietin is present in the blood of animals with a normal complement of platelets and that its level is increased in those with thrombocytopenia. These results suggest that the normal rate of platelet production may be maintained by the constant stimulatory effect of thrombopoietin and support the concept that regulation is achieved by variation in the production of this hormone. It should be pointed out, however, that the site of production, stimulus for production, chemical nature, and mechanism of action of this proposed hormone are unknown.

In some rodents, erythropoiesis ceases completely if the hematocrit is raised to less than twice normal. In spite of production of much larger increments in platelet count, platelet production has persisted, at a reduced rate, even though thrombopoietin has not been demonstrable in the plasma of the animals with transfusion-induced thrombocytosis.<sup>3,4</sup> Thus, megakaryocytopoiesis may not be totally dependent on thrombopoietin. Alternatively, thrombopoietin production may not have been completely suppressed by the high platelet counts.

When platelet production is stimulated experimentally by acute depletion of circulating platelets, a number of changes occur endogenously. Megakaryocytes increase in size, ploidy, number, and maturation rate. The platelets produced are of increased size, and an increase in the rate of platelet production is

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*From St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Mass. 02135.*

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*Address for reprint requests: Shirley Ebbe, M.D., St. Elizabeth's Hospital, 736 Cambridge St., Brighton, Mass. 02135.*

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demonstrable from the platelet count and from the incorporation of radioisotopic tracers. If all of these changes were due to an elevated level of thrombopoietin, then all should be reproduced by passive transfer of the hormone. Early studies in which thrombopoietin was detected by its ability to increase the platelet count of recipient animals were found to lack reproducibility.<sup>5</sup> Most of the recent observations have depended on an increase in incorporation of <sup>35</sup>S-sodium sulfate or <sup>75</sup>Se-selenomethionine into platelets to detect passively transferred thrombopoietic activity. It is noteworthy that increases in incorporation are usually not associated with parallel increases in platelet counts of the animals used for bioassay. Thus, the current techniques for detecting thrombopoietin appear to be measuring a single component of the endogenous response to stimulation, namely, an increase in platelet size. Systematic analysis of the effects of passively transferred thrombopoietin on megakaryocytes has not been reported, and the megakaryocyte adaptation that may specifically lead to the increase in platelet size has not been identified. Platelet production may also be modestly increased endogenously by the trauma associated with surgical procedures which results in the so-called reactive thrombocytosis. In that case, megakaryocyte maturation time is reduced, but megakaryocyte size is normal; the platelets, too, have been found to be of normal size.<sup>6</sup> These findings imply that macromegakaryocytosis may be responsible for the increase in platelet size, and if so, it should be demonstrable in the recipients of thrombopoietin.

The occurrence of reactive thrombocytosis in response to a nonthrombocytopenic stimulus is one of the major obstacles to a satisfactory unified concept about thrombocytopoiesis revolving about a hormonal thrombopoietin. It has been proposed that a specific response to platelet number may produce changes in megakaryocyte ploidy and size and that other regulators account for the increase in the number of megakaryocytes found with reactive thrombocytosis.<sup>7</sup> However, the occurrence of all these changes together (increase in ploidy, size, number, and maturation rate) in the thrombocytopenic animal that has been subjected to a minimum of trauma supports the alternative possibility that a single humoral agent may mediate all of the responses with the degree and type of response dependent on its concentration. Recent observations suggest that the thrombopoietic activity of plasma from thrombocytopenic animals may be concentrated by fractionation techniques.<sup>8,9</sup> If these techniques result in a potent preparation of thrombopoietin which would permit maximal and graded degrees of stimulation of thrombocytopoiesis by an exogenous source, some of these problems would be solved.

There are other findings that do not fit with a unified concept that platelet production is controlled solely by the level of thrombopoietin which is, in turn, determined by the concentration of circulating platelets. A number of observations have been reported in which platelet survival has been modestly prolonged, for example, by anticoagulants, or shortened by intravascular coagulation or prosthetic devices. The result has not been alteration of the platelet count, but, rather, an adjustment of platelet production. Compensated thrombolytic states have also been described in human beings as a result of immune destruction of platelets.<sup>10</sup> These findings clearly suggest that the rate of

peripheral destruction of platelets may influence platelet production independently of the platelet count.

In contrast, clinical and experimental hypersplenism is associated with thrombocytopenia largely due to splenic sequestration of platelets, with the total extramedullary complement of platelets (circulating plus splenic) remaining about normal.<sup>11</sup> Megakaryocytopoiesis may be modestly stimulated, but, if a normal platelet count in the peripheral blood is the important end point of the action of thrombopoietin, then it seems curious that the moderate degrees of thrombocytopenia associated with hypersplenism are not corrected by a cell system that appears to have the capability to increase cell production several-fold and a humoral regulator that should be sensitive enough to maintain normal platelet levels without wide fluctuations. The total body complement of extramedullary platelets may, therefore, be a more important determinant of humoral regulation of platelet production than the concentration of platelets, suggesting the possibility that some absorptive (i.e., serotonin) or adsorptive (i.e., plasma proteins) function may be concerned in thrombopoietic regulatory mechanisms.

Currently available data, therefore, indicate that megakaryocytopoiesis and platelet production are responsive to alterations in the peripheral platelet count and that some of the responses may be mediated by a humoral substance that has been called thrombopoietin. The data do not permit invoking this mechanism to explain all of the adjustments in platelet turnover that have been described. Some of the apparent inconsistencies might be clarified if a critical platelet function responsible for regulating thrombopoietin production could be identified.

Platelets participate in primary hemostasis, but the normal number of circulating platelets is in excess of that necessary to maintain hemostasis, so this function seems an unlikely one to regulate a sensitive homeostatic mechanism. Further, patients with intrinsically dysfunctional platelets do not develop thrombocytosis. Platelets may interact with a variety of foreign or pathologic materials<sup>12</sup> and may participate in inflammatory reactions.<sup>13</sup> The occurrence of thrombocytosis in association with tissue injury, chronic blood loss, and some inflammatory and malignant diseases is probably not a nonspecific reaction. It suggests that there is an increased demand for one or more of the functional capabilities of platelets in these disorders. The thrombosthenin in the platelet has been likened to the hemoglobin in red cells, namely, a tissue-specific protein component which may exert some control over megakaryocytic differentiation<sup>14</sup> and may be the platelet constituent that is monitored to regulate thrombopoietin production.<sup>15</sup> The cellular mechanisms by which thrombosthenin or any other platelet constituent may be monitored by a tissue which would produce thrombopoietin remain to be established and are conceptually elusive.

In addition to long-range regulation of platelet production, it is likely that short-range factors related to intercellular reactions or microenvironmental influences are capable of stimulating megakaryocytopoiesis. Recent observations with the cytotoxic drug vincristine<sup>16</sup> and with  $W/W^v$  or  $Sl/SI^d$  mice suggest that reduced numbers of megakaryocytes themselves or abnormalities of their environment may produce compensatory changes in megakaryocyto-

poiesis. The possible role of induction of short-range factors in the mediation of feedback mechanisms that originate in the periphery is unexplored.

Human thrombocytopoiesis has been found to show many of the same reactions as have been observed in experimental animals. Therefore, elucidation of the regulatory mechanisms in laboratory animals should yield information that is applicable to the role of these mechanisms in human disease. In the meantime, it would seem prudent to keep an open mind about thrombopoietin and to look for other regulatory mechanisms or a platelet function that would unify the several types of thrombocytopoietic stimulation into a single mechanism.

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