expressing IL-4R in B-CLL patients was significantly higher in patients than in controls ($P < .01$). The proportion of CD3$^+$ cells expressing IL-4R in B-CLL patients was significantly higher in patients than in controls ($P < .03$ and $P < .01$, respectively). The proportion of CD19 and CD3$^+$ cells expressing cytoplasmic IL-4 was also significantly higher in B-CLL patients than in controls ($P < .03$ and $P < .01$, respectively).

In this model, Tpo$^{+}$ cells constitutively produced and released in the circulation, where it is being cleared by platelets through c-mpl–mediated binding. But, to date, the site(s) of Tpo production remains to be defined. Using Northern blot analysis, the liver and kidneys appear to be the major site of Tpo mRNA expression. However, using more sensitive methods, such as reverse transcription-polymerase chain reaction (RT-PCR), Tpo transcripts can be detected in most tissues, including the bone marrow stroma, which could produce Tpo directly in the megakaryocyte microenvironment. To evaluate the contribution of the liver to the Tpo production required to maintain a normal platelet count, we have generated tissue-specific knockout mice by transplanting the liver of Tpo-deficient mice into wild-type recipients. As shown in Fig 1A, transplanted with a Tpo-KO liver, the Tpo transcripts were absent from the liver only. Circulating Tpo levels were assessed by a c-Mpl–dependent proliferation assay, which could be detected in the megakaryocyte microenvironment. To evaluate the contribution of the liver to the Tpo production required to maintain a normal platelet count, we have generated tissue-specific knockout mice by transplanting the liver of Tpo-deficient mice into wild-type recipients. As shown in Fig 1A, transplanted with a Tpo-KO liver, the Tpo transcripts were absent from the liver only. Circulating Tpo levels were assessed by a c-Mpl–dependent proliferation assay, which could be detected in the megakaryocyte microenvironment.

Table 1. Comparison of Cytoplasmic IL-4 and IL-4R Expression in B-CLL Patients Versus Controls

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CD19/IL-4 (pg/mL) Unst*</th>
<th>CD3/IL-4 (pg/mL) Unst*</th>
<th>CD3/ML-4 (pg/mL) Spon</th>
<th>CD3/ML-4 (pg/mL) PHA</th>
<th>Controls CD19/IL-4 (pg/mL) Unst*</th>
<th>CD3/IL-4 (pg/mL) Unst*</th>
<th>CD3/ML-4 (pg/mL) Spon</th>
<th>CD3/ML-4 (pg/mL) PHA</th>
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<td>21.57</td>
<td>97.13</td>
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<td>11.22</td>
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<td>10.13</td>
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<td>10</td>
<td>8.32</td>
<td>0.93</td>
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Abbreviation: unst, unstimulated.

*The data are presented as a percentage of CD19 and CD3$^+$ cells expressing cytoplasmic IL-4 and surface IL-4R. The proportion of CD3$^+$ cells expressing IL-4R in B-CLL patients was significantly higher in patients than in controls ($P < .01$). The proportion of CD19 and CD3$^+$ cells expressing cytoplasmic IL-4 was also significantly higher in B-CLL patients than in controls ($P < .03$ and $P < .01$, respectively).

Primary Role of the Liver in Thrombopoietin Production Shown by Tissue-Specific Knockout

To the Editor:

Maintenance of an appropriate number of circulating platelets is ably important in the pathogenesis of B-CLL and merits further investigation.

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Table 2. IL-4 Secretion by Lymphocytes From B-CLL Patients and Normal Controls

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Spon (pg/mL)</th>
<th>PWM (pg/mL) Controls</th>
<th>Spon (pg/mL)</th>
<th>PWM (pg/mL)</th>
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<tbody>
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<td>ND</td>
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<tr>
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</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>ND</td>
<td>7</td>
<td>ND</td>
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</tbody>
</table>

IL-4 secretion by lymphocytes from normal controls was significantly increased compared with that from the patients ($P < .01$).

Abbreviations: Spon, spontaneous secretion; PHA, phytohemagglutinin-stimulated secretion; PWM, pokeweed mitogen-stimulated secretion; ND, not detected.

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and transplanted animals (data not shown). Blood cell counts analysis up to 12 weeks after the operation indicated that the circulating platelet levels of the transplanted mice were intermediate between those of normal and Tpo-KO mice, suggesting that the liver contributes approximately 60% of the Tpo required for maintenance of a normal platelet count (Fig 1B). All other blood parameters analyzed, including red blood cells and total white blood cells, were normal in animals transplanted with a wild-type or Tpo-KO liver.

Thrombocytopenia associated with liver cirrhosis has historically been attributed to hypersplenism; however, lower than normal platelet counts seem to persist in many patients, even after splenectomy or portal decompression. The present results suggest that thrombocytopenia associated with liver disease such as cirrhosis may be attributable to reduced Tpo production. This is consistent with the finding that Tpo levels in these patients are not elevated even with their reduced platelet count, suggesting a potential defect in Tpo production. We have previously shown using quantitative PCR that Tpo mRNA levels were reduced in cirrhotic liver. The reduction in Tpo production may not be restricted to decrease of mRNA but may be combined with a posttranscriptional decrease in active protein, as has been documented for other liver-derived proteins. Our data demonstrate that the liver is the major site of Tpo production and that altered hepatic Tpo production will lead to a significant reduction in platelet levels. Secondary sites of Tpo production are likely to include the kidneys, which express significant amount of Tpo mRNA and are also responsible for erythropoietin production. The bone marrow stroma could provide part of the remaining Tpo, because it would be directly delivered in the megakaryocyte microenvironment.

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Fig 1. RT-PCR analysis and platelet counts in liver specific Tpo-KO mice. Liver from Tpo-KO mice or wild-type littermates were transplanted in normal recipients, as previously described. (A) Tpo mRNA expression was analyzed in the kidney (K), liver (L), muscle (M), and spleen (S) by RT-PCR. (B) Platelet counts were measured from retroorbital bleeds 12 weeks after transplant; n = 6 mice per group.
CORRESPONDENCE


The Pathway of Exocytosis in Human Platelets

To the Editor:

In a report titled “Regulated Secretion in Platelets: Identification of Elements of the Platelet Exocytosis Machinery,” recently published by Lemons et al in Blood, the authors have investigated the molecular events involved in membrane fusion in platelets. Therein, the function of molecules mediating the secretory events of the exocytosis in platelets was attributed to membranes of structures that might be involved in this process. There are no doubts or any objection to the biochemical results of the article. On the other hand, the investigators discuss their original findings on a critical morphological basis. In the introduction they wrote, “In one model of platelet activation,2-4 these stimuli (collagen, thrombin, and ADP) trigger morphological changes in the platelet resulting in the apparent movement of the secretory granules to the center of the cell and their subsequent fusion with the surface connected canalicular system (SCCS).” The disadvantage of this model is that the fusion of membranes of secretory organelles with the membranes of the SCCS has never been demonstrated.5,6 In contrast, it was clearly shown in studies, using rapid freezing with a time resolution in the range of milliseconds to capture fusion events,7 that the secretory organelles (α-granules2,6 and dense core bodies8,10) fuse with the plasma membrane when the platelets were stimulated before. The fusion of secretory organelles with the plasma membrane in human platelets is a phenomenon seen in secretory cells in general.8,11 From morphological observations8,12 and morphometrical measurements,12,13 it was concluded that the fate of the SCCS during stimulation is to become evaginated within seconds to allow the surface enlargement necessary for formation of pseudopodia or spreading. Fully stimulated platelets do not show SCCS but do show the membranes of degranulating organelles.8,10,14 As a consequence, thrombin-stimulated gray platelets, which lack α-granules, do not contain such membrane convolutes.14

Furthermore, the investigators wrote that platelet granule membranes do not appear to be docked, in comparison with synaptic vesicles. Interestingly, α-granules and also dense core granules dock before or at the beginning of stimulation to the plasma membrane. In activated platelets after organelle centralization by the action of the contractile cytoskeleton, the membranes of both types of secretory organelles show docking.8,10 Docking, also named apposition, is longer-lasting than membrane fusion and stable enough to be demonstrable with chemical fixation.8,15,16 Granules in apposition with the plasma membrane maintain this position during subsequent shape change and internal contraction. These reactions progress in the time range of seconds. An investigation using the atomic force microscope on living platelets gave support to this view of platelet exocytosis.17 Secretory organelles that are apposed to the surface membrane (and arrested there) fuse and induce sequential fusion and the formation of compound granules. The constriction of the contractile cytoskeleton in platelets moves (mobile) platelet organelles to the platelet center and into contact with each other, supporting further apposition and leading to formation of compound granules.6,11 It should be noted that the formation of compound granules during platelet secretion was already suggested from immunocytochemical and morphological investigations by Ginsberg et al in 1980.18

From their results, Lemons et al suggested that the molecular mechanism working in platelets for exocytosis is similar to that one described in other cells, most notably neurosecretory cells. I would like to add the suggestion that the compound exocytosis of platelets corresponds to that one of other secretory cells, most likely mast cells.19,20 The attribution of regulatory molecules with distinct functions in exocytosis to certain membranes should reflect the described secretory pathway in platelets.

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Primary Role of the Liver in Thrombopoietin Production Shown by Tissue-Specific Knockout

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