P-Selectin, and Not E-Selectin, Negatively Regulates Murine Megakaryocytopoiesis

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P-Selectin, and Not E-Selectin, Negatively Regulates Murine Megakaryocytopoiesis

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To assess the role of P-selectin and E-selectin in megakaryocytopoiesis, in vitro assays were performed in animal models deficient in both adhesion receptors. There was a significantly greater number of IL-3-responsive megakaryocyte progenitors CFU (CFU-MK) and an increase in immature megakaryoblasts in response to IL-6 in the P-selectin-null mice compared with the wild-type controls. Furthermore, P-selectin-null mice showed a greater number of CFU-MK colonies derived from CD34+ cells in response to IL-3 or IL-3 plus stem cell factor. A significant shift in baseline ploidly with a reduction in 8N cells and an increase in 32N cells was also observed in the P-selectin-null mice. Secretion of the inhibitory growth factor TGF-β1 and not TGF-β2 was significantly lower in the supernatants of cultures containing bone marrow cells from P-selectin-deficient mice as compared with those from the wild-type control bone marrow cells. No differences in the responsiveness of murine CFU-MK, immature megakaryocytes, or 5-fluorouracil-selected stem cells to cytokines were observed in E-selectin-null mice as compared with the control mice. These studies indicate that the absence of P-selectin, and not E-selectin, resulted in an altered adhesion environment with subsequent expansion of megakaryocyte progenitors and immature megakaryoblasts, enhanced secretion of TGF-β1, and apparent increased responsiveness to inflammatory cytokines. The Journal of Immunology, 2002, 169: 4579–4585.

Cellular interactions between hematopoietic stem cells and stromal cells play a critical role in the regulation of hematopoiesis. These cellular adhesion interactions serve multiple functions ranging from the self renewal of primitive hematopoietic stem cells within the bone marrow to the differentiation and release of mature hematopoietic cells into circulation. In addition, such interactions may be responsible for the homing of hematopoietic stem cells to the marrow after bone marrow transplantation, endogenous migration, or the homing to extramedullary sites in the disease state. Hematopoietic cells express different adhesion receptors in conjunction with specific stages of differentiation and maturation, and a variety of cellular adhesion molecules are involved in mediating a range of interactions throughout hematopoiesis (1, 2). These adhesion molecules belong to three different families: selectins, integrins, and Igs.

The stimulation of platelet production and the regulation of megakaryocytopoiesis are modulated by two mechanisms: a physiological one with terminal maturation mediated by thrombospondin (TPO) and an inflammatory one mediated through cytokines such as IL-6 and IL-3. The adhesive interactions of megakaryocytes with other vascular cells are essential in modulating the growth and function of cells of this lineage. P-selectin, a member of the selectin family of adhesion molecules, is an integral membrane protein found in the α-granules of platelets and the weibel palade bodies of endothelial cells. Upon stimulation of platelets and endothelial cells with a variety of agonists such as thrombin, histamine, and the calcium ionophore A23187, P-selectin is phosphorylated and rapidly translocated to the plasma membrane, where it mediates leukocyte-platelet and leukocyte-endothelial cell adhesion. E-selectin, which is specific to endothelial cells, also mediates leukocytes. In contrast to P-selectin, E-selectin is synthesized by the endothelium only after exposure to inflammatory cytokines and is not stored (3). Activated platelets have also been shown to secrete P-selectin. Both platelets and endothelial cells contain mRNA encoding the soluble form of P-selectin. P-selectin contains lectin that binds to the mucin-like P-selectin glycoprotein ligand-1, expressed on leukocytes of the myeloid lineage (4, 5). The interactions of P-selectin with leukocytes lead to the capture of leukocytes on the vascular surface, as demonstrated in vitro (6–8) and in vivo experiments (9) and by the induction of tissue factor on monocytes (10). P-selectin has also been reported to function as a cell adhesion molecule for leukocyte precursor cells in the bone marrow, including both lineage-restricted clonogenic progenitors (granulocyte-macrophage CFU (CFU-GM) and burst-forming unit erythroid (BFU-E)). Primitive hematopoietic progenitor cells have been reported to bind P-selectin and express P-selectin glycoprotein ligand-1.

The biological relevance of platelet P-selectin, a specific marker of platelet activation, is still unknown. It has been shown that platelets roll on stimulated endothelium in vivo, but this is mediated by endothelial cells and not by platelet P-selectin. Platelets collected from mice deficient for P-selectin were found to be non-functional, and megakaryocyte counts were high in the mice marrow (11). Mice genetically engineered with a deficiency in P-selectin, as well as mice doubly deficient in P- and E-selectins generated by two rounds of homologous recombination, exhibit deficits in leukocyte rolling and extravasation. E-selectin-deficient mice show no obvious defects in leukocyte extravasation, although...
the addition of P-selectin Ab leads to reduced leukocyte influx in thioglycolate-induced peritonitis and to delayed-type hypersensitivity reaction. Although very little is known about the involvement of adhesion molecules in megakaryocytopenia, it has been suggested that the regulation of both megakaryocytopenia and platelet production involves the interplay of hematopoietic growth factors and cell-cell adhesive interactions within the bone marrow environment (12, 13). Prior studies from our laboratory showed that megakaryocyte growth was promoted after adhesion to marrow stromal fibroblasts, and this phenomenon was mediated in part through the transmembrane form of c-kit ligand (14). It was also shown that adhesion of megakaryocytes to endothelial cells resulted in the enhancement of the maturation process of the megakaryocytes (15–17). Platelet formation was found to be enhanced by the transendothelial migration of megakaryocytes in response to the chemokine stromal cell-derived factor-1 (18). All of these studies suggest that, during extension of the cytoplasmic process in vivo, the interaction between megakaryocytes and endothelial cells is important. It was suggested that platelet endothelial cell adhesion molecule-1 may be involved in the cellular interaction between mature megakaryocytes and endothelial cells during platelet formation (19). P-selectin was found to be expressed only in the later stages of maturing megakaryocytes (14, 20, 21).

In this report, we characterized the role of P- and E-selectins in the regulation of megakaryocytopenia. Our study indicates that the absence of P-selectin resulted in an altered adhesion environment in the bone marrow with an expansion of megakaryocyte progenitors and immature megakaryocytes. Furthermore, the data suggest that P-selectin negatively regulates megakaryocytopenia through enhanced secretion of the inhibitory growth factor TGF-β1.

Materials and Methods

**Mice**

P-selectin-, E-selectin-, and both P- and E-selectin-deficient mice were derived as described previously (11, 22). These mice were kindly obtained from Dr. D. D. Wagner (Center for Blood Research, Boston, MA). Experimental mice were 7- to 12-wk-old male (19–30 g) age-matched P-selectin-deficient and wild-type mice. In addition, age-matched E-selectin-deficient mice and wild-type mice were also used. Animals were bred and maintained in a virus Ab-free facility at the Longwood Medical Center and Center for Blood Research (Harvard Medical School, Boston, MA) and in a specific pathogen-free animal facility at the Harvard Institutes of Medicine (Beth Israel Deaconess Medical Center, Boston, MA). Generation and maintenance of the selectin-null and wild-type mice used in these studies were as described (11). P-selectin-null and wild-type mice were obtained from homozygous matings.

**Growth factors and Abs**

Recombinant murine IL-3, recombinant murine GM-CSF, human IL-6, recombinant stem cell factor (SCF), and recombinant erythropoietin (EPO) were obtained from R&D Systems (Minneapolis, MN). These cytokines were determined to be free of endotoxin contamination. Plateau doses of each factor were determined from dose-response curves for each assay. Recombinant murine TPO (mTPO; Genentech, South San Francisco, CA) was used at 100 ng/ml as determined from dose-response curves in the megakaryocyte progenitor assays (colony formation and liquid cultures). In some experiments, as indicated, we used various dilutions of TPO or IL-3 (10–100 ng/ml) to assess the synergistic effects of both cytokines, under conditions of subconcentration or optimum concentration, on the megakaryocytic lineage. Ab to P-selectin was obtained from BD Biosciences (San Jose, CA). Polyclonal antiserum to murine CD34⁺ cells was a gift from Dr. L. Lasky (Genentech).

**Isolation of CD34⁺ cells by the immunomagnetic bead technique**

CD34⁺ cells were isolated following the manufacturer’s instructions. Cells were first incubated at 4°C for 30 min with polyclonal antiserum to murine CD34⁺ and then with paramagnetic beads coupled with goat Ab to mouse IgG (Dynabeads M-450; Dynal Biotech, Great Neck, NY) with a bead: target cell ratio of 5:1. CD34⁺ cells were isolated by magnetic separation and detached from the beads by chymopapain treatment (130 U/ml for 10 min; Sigma-Alrich, St. Louis, MO), which allows for the collection of CD34⁺ cells free of beads.

** Colony assays**

BFU-E and CFU-GM were assayed using methyccellose medium with cytokines (IL-3, 20 ng/ml; EPO, 3 IU/ml; SCF, 50 ng/ml; StemCell Technologies, Vancouver, Canada) plus 0.5 ml of DMEM (2% FCS, 10 IU/ml penicillin, 10 μg/ml streptomycin, 1 mM L-glutamine). A total of 1.5 ml of this mixture was added to a 35-mm graduated petri dish. Duplicate assays were performed for each condition. Petri dishes were placed in a 5% CO₂ humidified incubator at 37°C for 10–21 days. After the incubation period, the number of CFU-GM colonies was determined by light microscopy (23). Positive colonies were scored on the basis of an accumulation of 40 or more cells. Three or more red cells clustered together were counted as a BFU-E colony (24). Counts were done in duplicate plates per assay.

** Murine megakaryocyte assay**

Mice were sacrificed by cervical dislocation and the femurs and tibiae were collected in PBS. Cells from the bone marrow were collected by flushing the bones with DMEM containing 10% FCS using a 1-ml syringe and a 25-gauge needle.

**Megakaryocyte colony assays**

Bone marrow cells (1 × 10⁶/ml) were plated in 35-mm petri dishes for megakaryocyte progenitor CFU (CFU-MK) with or without murine IL-3 or mTPO in 0.5% agar. After 10–12 days, the petri dishes were dried and stained for acetylcholinesterase. Three or more positive cells together were counted as a megakaryocyte colony as detected by light microscopy (25).

**Immature megakaryocyte assays.** To assess megakaryocytic differentiating activity, a single megakaryocyte growth assay was used (26). Single cell populations from bone marrow were prepared from the femurs of the mice. This preparation was performed by flushing the bones with DMEM containing 10% FCS. Immature megakaryocyte populations were obtained as a 1.07–1.085 g/cm³ fraction, from a suspension of single cells separated in a Percoll gradient (Sigma-Alrich). The fractionated cells were cultured in 10% FCS in DMEM for 5 days at 37°C in a 10% CO₂ humidified incubator. This procedure was performed in the presence of titrated doses of the growth factors mTPO and IL-6. In some cases, pretreated doses of Ab to P-selectin were added to the mTPO and incubated for 2 h at 4°C to neutralize the assays before culturing. An indirect immune complex depletion method was used as described (27). P-selectin Ab was incubated with mouse bone marrow enriched with immature megakaryocytes, in the presence or absence of TPO, for 2 h at 4°C. Cultures were dried and stained for acetylcholinesterase. Growth and maturation of immature megakaryocytes were quantitated by assessing the number of large single megakaryocytes detected by light microscopy.

**Flow cytometric analysis of surface protein expression**

Cells were washed with sterile PBS, and 1 × 10⁶ cells were resuspended in 0.1 ml of PBS. Cells were incubated with P-selectin Ab, GpIIa Abs, or mouse IgG as a control (Immunotech, Westbrook, ME) or with PBS at 4°C for 20 min. FITC-conjugated goat anti-mouse IgG or goat anti-rabbit IgG was added at a final dilution of 1:500 and followed by incubation for 20 min at 4°C. Cells were washed twice, resuspended in 0.5 ml of 1% (v/v) paraformaldehyde in PBS and then analyzed by flow cytometry. Flow cytometry was performed using a FACSCalibur flow cytometer (BD Biosciences). Appropriate controls of matched isotype Abs to establish positive and negative quadrants, as well as appropriate single color stains, were included.

**ELISA for cytokines**

Bone marrow cells from P-selectin-deficient mice and wild-type mice at 2 × 10⁶/ml were incubated in serum-free and cytokine-free cultures for 24, 48, and 72 h. Supernatants were collected from the cultures every 24 h. After centrifugation to remove cell debris, supernatants were aliquotted and frozen at −80°C until use. After the collection of all supernatants over a 3-day period, samples were thawed on ice and mixed well, and the concentrations of the secreted cytokines were measured with commercially available ELISA kits (Quantikine Immunoassay kits; R&D Systems) for mouse TGF-β1 and TGF-β2. Following the manufacturer’s instructions, reagents and working standards were prepared and assays were done. Briefly, diluents and 50–200 μl of supernatant or standard (depending on the particular assay) were added in duplicate into each well of the precoated microplates and allowed to incubate at room temperature for 2 h.
Control wells contained DMEM without serum and cytokine. The wells were then washed with wash buffer and the conjugate was added and allowed to incubate for 1.5–2 h. After washing, the substrate solution was added and allowed to incubate for 20–30 min. Finally, 50 μl of stop solution was added and the OD 450 was read immediately using the Multiskan Plus scanner set (Titertek, Huntsville, AL). A standard curve was established and cytokine concentrations (picograms per milliliter) were derived from the standard curve.

**Ploidy analysis of megakaryocytic cell lines**

Cells were plated in 24-well plates at 2 × 10^5/ml with 5% platelet-poor plasma for 5 days. Cells were then washed twice with HBSS and resuspended in nucleus isolation medium (0.2% BSA, 0.4% Nonidet P-40, and 10 mM HEPES (pH 7.4) in HBSS), and then 54 Worthington U/ml RNase A at 2 × 10^3/ml was added. An equal volume of nucleus isolation medium containing 25 μg/ml propidium iodide (Sigma-Aldrich) was then added. Samples were kept in the dark at 4°C and analyzed the same day on a FACScan using CellFit software (BD Biosciences).

**FIGURE 1.** Dose-dependent response of murine CFU-MK to IL-3 and TPO. A, Bone marrow cells (1 × 10^5/ml) from P-selectin-deficient mice (solid lines) and wild-type control mice (dashed lines) were cultured in the presence or absence of growth factors. Cultures were incubated for 8 days in humidified air containing 10% CO_2 at 37°C. Plates were dried and stained for acetylcholinesterase. Megakaryocyte colonies were counted as three or more acetylcholinesterase-positive cells. Results are the mean ± SD of five independent experiments. *, Statistically significant data points (p < 0.01). B, Responsiveness of CFU-MK colonies of P-selectin knockout mice to growth factors. CD34^+ marrow cells (4.5 × 10^5) were cultured in the presence or absence of the indicated growth factors. All of the growth factors were used at 50 ng/ml except mTPO, which was used at 100 ng/ml. Triplicate cultures for each treatment were analyzed and CFU-MK colonies were counted in each experiment. Results are the mean ± SD of five independent experiments. *, Results that are significantly different from wild-type mice.

**FIGURE 2.** Analysis of BFU-E and CFU-GM colonies in P-selectin knockout mice. A, Unfractionated bone marrow cells were plated per 3-mm petri dish at a density of 5 × 10^5 cells/ml in 1 ml culture medium with 0.9% methylcellulose. Dishes were cultured in the absence or presence of EPO (50 ng/ml) or EPO plus SCF (30 ng/ml) at 37°C in a 100% humidified atmosphere containing 5% CO_2. On day 14, red or pink aggregates of cells were scored as erythroid colonies. Duplicate cultures for each treatment were analyzed and BFU-E colonies were counted. Results are the mean ± SD of five independent experiments. B, Bone marrow cells (1 × 10^5/ml) were cultured in the absence or presence of GM-CSF, GM-CSF plus SCF, IL-3 plus IL-6 plus SCF, or SCF alone. After 10 days of culture at 37°C in a humidified atmosphere containing 10% CO_2, CFU-GM colonies were counted using standard criteria. All of the growth factors were used at a concentration of 50 ng/ml except SCF, which was used at 30 ng/ml. Triplicate cultures for each treatment were analyzed and CFU-GM colonies were counted. Results are the mean ± SD of five independent experiments.
Statistical analysis

The results are expressed as the mean ± SD of data obtained from five or more experiments performed in triplicate. Statistical significance was determined using the Student t test.

Results

Characterization of hematopoiesis in mice deficient in P-selectin and E-selectin

To assess hematopoiesis in P-selectin knockout mice, bone marrow cells were isolated from femurs and hematopoietic progenitor assays were performed. CFU-MK assays were performed with titrated doses of IL-3 and TPO (Fig. 1A). The responsiveness of megakaryocyte progenitors to IL-3 was three times (p < 0.01) higher in bone marrow cells isolated from P-selectin knockout mice as compared with those from wild-type mice. The response of progenitors to TPO was higher, but not significant, probably due to the fact that TPO has a less profound effect on early megakaryocyte progenitors than IL-3. The same responsiveness in megakaryocyte progenitors was seen in CD34+ cells isolated from the bone marrow of P-selectin-deficient mice (Fig. 1B). Other hematopoietic lineages were not found to be affected in the bone marrow cells from the P-selectin knockout mouse. As shown in Fig. 2, there was no effect on the responsiveness of BFU-E and CFU-GM colonies between the control mice and P-selectin knockout mice. Thus, megakaryocyte progenitors are significantly increased in the P-selectin-null mice as compared with the wild-type control mice.

Next, we analyzed the responsiveness of immature megakaryoblasts to growth factors that induce terminal differentiation of megakaryocytes, using the single cell assay. The responsiveness of immature megakaryocyte precursors to titrated doses of IL-6 and TPO was determined. There was a significant (p < 0.01) difference in the response of the immature megakaryocytes from P-selectin-deficient mice to IL-6 and TPO as compared with the response of the same cells from wild-type mice (p < 0.05) (Fig. 3). Interestingly, the response of immature megakaryocytes from P-selectin-deficient mice to high doses of TPO did not follow the normal pattern of feedback inhibition, as was observed in cells isolated from wild-type mice or in cells treated with IL-6.

The bone marrow cells from the E-selectin knockout mice did not show altered megakaryocytopoiesis (Fig. 4), as determined by a megakaryocyte progenitor assay and megakaryocyte maturation assay. Thus, P-selectin, and not E-selectin, regulates in vivo megakaryocytopoiesis.

Expression of P-selectin in TPO or IL-6 stimulated bone marrow populations enriched with immature megakaryocytes

TPO is a physiological regulator of normal megakaryocytopoiesis (28, 29). To analyze the expression of P-selectin on nonstimulated or

![FIGURE 3](http://www.jimmunol.org/)

FIGURE 3. Responsiveness of immature megakaryocyte precursors from P-selectin knockout mice. Percoll gradient-separated immature megakaryoblasts were seeded at 5 × 10⁴ cells/plate in the absence or presence of IL-6 or mTPO. After 5 days of incubation at 37°C in a humidified atmosphere containing 10% CO₂, the plates were stained for acetylcholinesterase to quantitate the mature megakaryocytes. Triplicate cultures at each time point were analyzed and the number of mature megakaryocytes was counted. Results are the mean ± SD of five independent experiments. The solid lines represent the number of megakaryocyte precursor cells obtained from the bone marrow cells of knockout mice and the dashed lines represent the number of megakaryocyte precursor cells obtained from the bone marrow cells of wild-type mice. *, Statistically significant data points (p < 0.01).

![FIGURE 4](http://www.jimmunol.org/)

FIGURE 4. A. Analysis of megakaryocyte progenitors in E-selectin knockout mice. Unfractionated bone marrow cells (1 × 10⁵) from E-selectin-deficient mice and wild-type mice were incubated in the absence or presence of IL-3 or mTPO for 8 days in a humidified atmosphere containing 10% CO₂. After staining for acetylcholinesterase, megakaryocyte colonies were counted as three or more cells. Results are the mean ± SD of five independent experiments. B. Megakaryocyte maturation in E-selectin knockout mice. Percoll gradient-separated cells (5 × 10⁵/ml) were incubated in the presence or absence of IL-6 or mTPO for 5 days in a humidified atmosphere containing 10% CO₂. Cells positive for acetylcholinesterase were counted as megakaryocytes. Triplicate cultures at each time point were analyzed and the number of mature megakaryocytes was counted. Results are the mean ± SD of five independent experiments.
stimulated bone marrow–derived megakaryocytes, bone marrow sub-
populations enriched with immature megakaryocytes were isolated
and cultured for 24 or 48 h with TPO or IL-6. The expression of
P-selectin increased with the maturation of megakaryocytes (Fig. 5).
Interestingly, there was also a significant shift in baseline ploidy with
a reduction in 8N cells and an increase in 32N cells in the P-selectin-
null mice as compared with the wild-type controls (Table I).

**Effect of P-selectin Ab on megakaryocytopoiesis is driven by TPO**

To evaluate the role of P-selectin in normal megakaryocytopoiesis,
P-selectin Ab was added to bone marrow cell cultures enriched
with immature megakaryocytes, in the presence or absence of
TPO, for 2 h at 4°C. Cells were analyzed in a semisolid
megakaryocytic growth assay, as described in Materials and Meth-
ods. The differentiation of megakaryocytes was analyzed by an
immature megakaryocyte assay. The response of immature
megakaryocytes in the presence of TPO was significantly higher in
the culture containing the P-selectin Ab, and not the control Ab,
suggesting that blocking the expression of P-selectin enhances the
regulatory pathway of megakaryocytopoiesis (Fig. 6A).

**Secretion of TGF-β cytokine by primary bone marrow cells from P-selectin-deficient mice**

TGF-β1, a multifunctional regulator of cell growth and develop-
ment, has been shown to inhibit all stages of the differentiation
process in the megakaryocytic lineage (30). The observation of
enhanced megakaryocytopoiesis in P-selectin-deficient mice sug-
gests that P-selectin might be involved in the secretion of TGF-β
by the bone marrow cells. Using TGF-β cytokine assays, super-
natants of cultures containing bone marrow cells from P-selectin-
deficient and wild-type mice were assayed at different time inter-
vals for the synthesis and secretion of TGF-β1 and TGF-β2.

**Table 1. Marrow megakaryocyte ploidy analysis**

<table>
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<tr>
<th>Cells</th>
<th>DNA Content</th>
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<tr>
<td></td>
<td>2N</td>
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<tr>
<td>P-selectin knockout mice</td>
<td>3.5</td>
</tr>
<tr>
<td>Wild-type mice</td>
<td>2.5</td>
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* DNA content of megakaryocyte ploidy was determined by staining with pro-
pidium iodide and analyzed by FACS. This is a representative experiment performed
three times.
TGF-β1 secretion was significantly lower in the supernatant of cultures containing bone marrow cells from P-selectin-deficient mice at 48 h of incubation as compared with its level of secretion in cells from the wild-type control (Fig. 6B). No changes were observed in TGF-β2 secretion (data not shown). Thus, P-selectin may negatively regulate megakaryocytopoiesis by regulating the secretion of TGF-β1, which down-regulates megakaryocyte development and maturation.

Discussion

These studies relate to the understanding of megakaryocyte biology, particularly in the context of inflammatory diseases. It has been demonstrated in our laboratory that LFA-1/ICAM-1 is important for promoting megakaryocyte maturation upon interaction with the endothelium (15). To investigate the role of other adhesion molecules in megakaryocytopoiesis, mice deficient in either P-selectin or E-selectin were used in vitro assays. Two in vitro studies were performed using bone marrow cells from P-selectin-deficient mice and wild-type mice. By these two in vitro assays, megakaryocytopoiesis can be analyzed as two interconnected processes: 1) the CFU-MK assay in which the precursor cells proliferate into immature megakaryocytes and 2) the immature megakaryocyte growth assay (differentiation assay) in which the immature megakaryocytes go into a process of development and maturation into fully developed megakaryocytes ready to shed platelets.

We observed a significantly greater number of IL-3-responsive CFU-MK in the P-selectin-deficient mice as compared with the wild-type control mice. Similar results were obtained with CD34+ cells isolated from the bone marrow of P-selectin-deficient or wild-type mice. A significant increase in the maturation of megakaryocytes in response to IL-6 and TPO was observed in the P-selectin-deficient mice. Other hematopoietic systems were found to be unaffected in the bone marrow cells of P-selectin-deficient mice and wild-type control mice. To evaluate whether P-selectin might affect the secretion of cytokines that modulate megakaryocytopoiesis, bone marrow cells (10^7/ml) were isolated from P-selectin-deficient and wild-type control mice and were analyzed for the secretion of TGF-β1 and TGF-β2. Interestingly, the level of TGF-β1 secretion was significantly lower when compared with the level of secretion from the control mice. TGF-β1 has been shown to inhibit the development of most early hematopoietic progenitors in vitro. TGF-β1 can simultaneously augment and suppress distinct cell lineages in peripheral and central hemopoietic systems. TGF-β was reported to have specific effects on the developmental stages of murine megakaryocytopoiesis. In vivo and in vitro experiments demonstrated inhibitory effects of TGF-β on megakaryocytopoiesis, which were mediated through secondary cytokines and receptors (30, 31). TGF-β1 can differentially regulate multiple hematopoietic pathways in a systemic, reversible, and dose-dependent fashion. TGF-β1 systemically modulates granuloid, erythroid, lymphoid, and thrombocytic cells in mice (31). These actions may be mediated by the direct effects of TGF-β1 or through modulation of secondary cytokines and receptors. Thus, the effects observed in the P-selectin mice, as described in this study, suggest that P-selectin may negatively modulate megakaryocytopoiesis by enhancing the secretion of TGF-β1, which down-regulates megakaryocyte development and maturation.

No differences were observed in the number of CFU-MK colonies or in the development of immature megakaryocytes in the E-selectin-deficient mice. These studies indicate that the absence of P-selectin, but not E-selectin, results in an altered adhesion environment and expansion of megakaryocyte progenitors and immature megakaryoblasts. These data also suggest that P-selectin negatively regulates megakaryocytopoiesis via the secretion of TGF-β1 from the cells.

We have also previously analyzed megakaryocytopoiesis in ICAM-2-deficient mice. We found a significant decrease in the number of IL-3-responsive CFU-MK colonies when compared...
with the control mice (32). Significantly fewer mature megakaryocytes were obtained from the bone marrow cells of the ICAM-2-deficient mice (32). The altered megakaryocytopoiesis observed in ICAM-2-deficient mice indicates that ICAM-2 is also an important molecule in megakaryocyte development.

Surface adhesion molecules such as P-selectin and LFA-1 are important mediators of megakaryocyte adhesion to HUVEC and/or marrow stromal macrophages or fibroblasts, and these adhesive interactions contribute to the regulation of megakaryocyte growth and maturation. Furthermore, inflammatory stimuli known to upregulate P-selectin expression in endothelium are likely to have similar effects in marrow megakaryocytes, providing a possible mechanism whereby megakaryocyte and platelet function are modulated in the context of the inflammatory response. It is possible that ligand binding to P-selectin or activation of LFA-1 leads to the induction of inflammatory cytokines (IL-1β, TNF-α) and/or growth factors (GM-CSF, IL-3, IL-6) from marrow megakaryocytes.

This suggests an autocrine mechanism of augmented megakaryocytopoiesis and thrombopoiesis during inflammation.

Taken together, these studies suggest that adhesion molecules participate directly in the homing, proliferation, and differentiation of hematopoietic cells, and that the absence of these molecules might lead to either a primary or a secondary dysregulation of hematopoiesis and/or megakaryocytopoiesis.

Acknowledgments

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References