RAPID COMMUNICATION

Chronic Exposure to Retroviral Vector Encoded MGDF (mpl-Ligand) Induces Lineage-Specific Growth and Differentiation of Megakaryocytes in Mice

By Xiao-Qiang Yan, David Lacey, Frederick Fletcher, Cynthia Hartley, Patricia McElroy, Yu Sun, Min Xia, Sharon Mu, Chris Saris, David Hill, Robert G. Hawley, and Ian K. McNiece

Megakaryocyte growth and development factor (MGDF) has recently been identified as a ligand for the c-mpl receptor. Using retroviral-mediated gene transfer, MGDF has been overexpressed in mice to evaluate the systematic effects due to chronic exposure to this growth factor. MGDF overexpressing mice had more rapid platelet recovery than control mice after transplantation. Following this recovery, the platelet levels continued increasing to fourfold to eightfold above normal baseline levels and remained elevated (fivefold above control mice) in these animals, which are alive and well at more than 4 months posttransplantation. Increased megakaryocyte numbers were detected in a number of organs in these mice including bone marrow, spleen, liver, and lymph nodes. Prolonged overexpression of MGDF led to decreased marrow hematopoiesis, especially erythropoiesis, with a shift to extramedullary hematopoiesis in the spleen and liver. All the MGDF overexpressing mice analyzed to date developed myelofibrosis and osteosclerosis, possibly induced by megakaryocyte and platelet produced cytokines. No significant effect on other hematopoietic lineages was seen in the MGDF overexpressing mice, showing that the stimulatory effect of MGDF in vivo is restricted to the megakaryocyte lineage.

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Colony-forming unit-erythroid (CFU-E) colonies were scored at day 9 methyl cellulose cultures and processed individually. DNA was fractionated by electrophoresis in a formaldehyde/agarose gel and transferred onto nylon membranes (Hybri-N +; Amersham, Arlington Heights, IL). Membranes were prehybridized in a 50% buffer (50 mM Tris, 0.1% SDS, 1% xylene crotonate solution (4.5 mol/L). GTCC, 50 mM/L sodium succinate (pH 7.0), 0.5% (v/v) sodium sarcosyl, 5% (v/v) xylene crotonate, and 5% (v/v) sodium succinate (pH 7.0) were used as a blocking solution. The blots were then incubated with the specific probe at 50°C for 20 hours. After the incubation, the blots were washed three times with 0.1× SSC and 0.1% SDS at 65°C, and then hybridized with a mixture of probes containing a full-length murine c-mpl cDNA sequence and are dependent on the presence of either murine IL-3 or MGDF to survive in culture. One unit of MGDF activity is defined as the amount required to support 20% viability of 32D/Mpl+ cells after 2 days of incubation in the absence of mIL3, at an initial inoculum of 1,000 cells per Terasaki microtiter well. One unit of MGDF activity is approximately equal to 0.5 pg of purified recombinant MGDF protein in this assay.

Bio-Assay for MGDF

MGDF activity in platelet-poor plasma and spleen conditioned medium of reconstituted animals was measured by 32D/Mpl+ assay as described previously.9 32D/Mpl+ cells were engineered to carry the full-length murine c-mpl cDNA sequence and are dependent on the presence of either murine IL-3 or MGDF to survive in culture. One unit of MGDF activity is defined as the amount required to support 20% viability of 32D/Mpl+ cells after 2 days of incubation in the absence of mIL3, at an initial inoculum of 1,000 cells per Terasaki microtiter well. One unit of MGDF activity is approximately equal to 0.5 pg of purified recombinant MGDF protein in this assay.

Histological Analysis

For histologic analysis, MGDF overexpressing mice (n = 10), killed at 7 and 10 weeks after BMT, were assessed. The control groups included normal, age-matched, nontransplanted mice (n = 5), mice transplanted with nontransfected BM (n = 5), and mice transplanted with BM cells transfected with the MSCV vector sequence alone (n = 10). Unless otherwise noted in the text, control mice refers to those animals transplanted with BM infected with the MSCV vector alone. Whole bone radiographs were obtained on two control (MSCV vector) and three MGDF overexpressing mice at 11 weeks posttransplantation using a Faxitron X-ray cabinet (Buffalo Grove, IL) and Kodak X-OMAT-TL film.

For standard organ histology, tissues (brain, lung, heart, thymus, spleen, liver, pancreas, trachea, esophagus, stomach, small and large intestine, ovaries, uterus, adrenal gland, kidney, bladder, bone, BM, skin, and skeletal muscle) were fixed in zinc formalin. For bone and BM histology, femurs were decalcified using an 8 N formic acid:1 N sodium formate (1:1) solution after formalin fixation. The tissues were then processed using standard histologic methods to yield 3-μm, paraffin sections that were subsequently stained with hematoxylin and eosin. The bone sections were analyzed histochemically using Masson’s trichrome and Gomori’s reticulum stains. Enzyme histochemistry for trurrate resistant acid phosphatase was performed using a kit (Sigma kit no. 387-A; St Louis, MO). To enhance the identification of megakaryocytes and platelets, immunohistochemistry for von Willebrand factor (vWF; Dako, Carpenteria, CA) was also performed using an autostained immunostain (Biotek, Santa Barbara, CA).

RESULTS

Generation of MGDF Overexpressing Mice

Four-day post-5-FU murine BM cells were cultured in the presence of recombinant retroviral particles, as described in Materials and Methods. Parallel cultures were infected with virus derived from the parental vector, MSCV, or the MSCV vector that also expressed murine MGDF cDNA from the viral LTR (Fig 1). The efficiency of infection of BM precursors was determined by colony assay in the presence and
The retroviral expression of MGDF (mpl-L) was assayed in mice lethal irradiated, syngeneic recipient mice were transplanted with vector-infected cells. Expression of vector-encoded MGDF, and the consequence of chronic MGDF overexpression was assessed after transplantation of infected BM cells, peripheral blood (PB) was analyzed weekly. MGDF overexpression was evaluated and all had elevated platelet counts, ranging from fourfold to eightfold higher than control mice. None of the 55 control mice had increased platelets. Normal function was shown for platelets from MGDF overexpressing mice using a thrombin-dependent platelet clotting assay (data not shown).

In vivo reconstitution of hematopoiesis with provirus-containing cells was assayed at 7 weeks posttransplantation by PCR analysis for the vector-encoded neo gene in GM-CFC cultured from the BM, spleen, and peripheral blood (PB) of recipient animals. In control recipients, 65% of GM-CFC from the BM contained the neo gene indicative of provirus integration. In MGDF overexpressing mice, 73% of GM-CFC from the BM; 80% of GM-CFC from the spleen and 81% of GM-CFC from the PB were positive for the neo gene.

Northern analysis of total RNA prepared from spleen cells and BM cells of recipient animals (7 weeks posttransplantation) showed the presence of the expected 4.5-kb MGDF mRNA transcript in the MGDF overexpressing mice, whereas the control mice had no detectable MGDF mRNA expression (Fig 2). Functional MGDF protein was assayed in the plasma of recipient animals as a function of time posttransplantation, using the 32D/mpl+ bioassay.9 No MGDF activity was detected in the plasma of animals before irradiation and transplantation, but detectable levels were observed in all animals posttransplantation (Fig 3A). MGDF levels in MGDF overexpressing mice were 15-fold and 27-fold increased compared with control animals at day 7 and day 14 posttransplantation, respectively. Plasma MGDF levels decreased sharply after day 7 and were undetectable after day 28 posttransplantation. However, conditioned medium harvested from cultured 10-week posttransplantation spleen cells (from MGDF overexpressing mice) had 52-fold increased MGDF bioactivity compared to control cells (Fig 3B). The MGDF activity in the spleen cell conditioned medium was completely neutralized by addition of soluble mpl receptor (Fig 3B), demonstrating the specificity of this bioassay and the production of functional MGDF protein in MGDF overexpressing mice at 10 weeks posttransplantation.

Thrombocytosis in MGDF Overexpressing Mice

After transplantation of infected BM cells, peripheral blood (PB) was analyzed weekly. MGDF overexpressing mice had rapid platelet recovery after transplantation and reached preirradiation levels at about 10 days (Fig 4A). In contrast, control mice required about 4 weeks for platelet levels to return to 80% preirradiation levels posttransplantation. Animals transplanted with uninfected BM reached the same platelet levels and with kinetics similar to the vector-infected controls (data not shown). Platelet counts continued to increase in the MGDF overexpressing mice and plateaued at 5 weeks posttransplantation at platelet counts of over 5,000 X 10^6/mL. These animals maintained elevated platelet counts over 4,000 X 10^6/mL for more than 16 weeks posttransplantation (data not shown). Control mice, after their return to normal preirradiation levels, maintained stable platelet levels for the duration of the experiment. In total, 117 MGDF overexpressing mice were evaluated and all had elevated platelet counts, ranging from fourfold to eightfold higher than control mice. None of the 55 control mice had increased platelets. Normal function was shown for platelets from MGDF overexpressing mice using a thrombin-dependent platelet clotting assay (data not shown). Red blood cell (RBC) counts in MGDF overexpressing mice tended to decrease after 3 weeks of reconstitution, with 20% to 30% reduction compared with control mice (Fig 4B). This decrease was associated with decreased hematocrit and hemoglobin concentration (data not shown).

Progenitor Cell Analysis of MGDF Overexpressing Mice

Two groups of mice were killed and analyzed at 7 and 10 weeks posttransplantation. Mice analyzed at 7 weeks received BM cells that underwent 3-day viral infection. Mice analyzed at 10 weeks received BM cells that had additional 2-day of G418 selection at the end of viral infection. Hematopoietic progenitor cells were examined in the BM, spleen, and PB of MGDF overexpressing mice and control mice (Table 3). CFU-E in the BM of MGDF overexpressing mice were greatly reduced 166-fold compared with control mice. An increase in the frequency of BFU-E, GM-CFC, CFChu, and MK-CFC was obtained in MGDF overexpressing mice. However, because of the reduced cellularity (4.6-fold decrease at 7 weeks posttransplantation), absolute numbers of these progenitor cells were decreased in the BM of the MGDF overexpressing mice compared with control mice. In contrast, spleens of MGDF overexpressing mice had elevated progenitor cells with 5.7-, 11-, 40-, 28-, and 8-9 methylcellulose cultures and analyzed by PCR with neo-specific and PDGF B receptor-specific primers. Results were from 5 mice killed at 7 weeks posttransplantation. The neo-positive colonies in the spleen and the PB of MSCV mice were not assessed because of low frequency of colony formation.

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**Table 1. Infection of NIH3T3 and BM Cells With Retroviral Vectors**

<table>
<thead>
<tr>
<th>Retroviral Vectors</th>
<th>NIH3T3 Infection</th>
<th>BM Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCV</td>
<td>G418 CFU/mL X 10^6 (n)</td>
<td>% of G418 total CFC (n)</td>
</tr>
<tr>
<td>MSCV</td>
<td>1.7 ± 0.9 (3)</td>
<td>55 ± 10 (4)</td>
</tr>
<tr>
<td>MGDF-MSCV</td>
<td>2.0 ± 0.5 (5)</td>
<td>52 ± 4 (5)</td>
</tr>
</tbody>
</table>

The titer of viral supernatant used in this study is shown as G418 resistant CFU per milliliter. For BM infection, cells were plated in methylcellulose culture with and without G418 at the end of infection. Plates were scored at 10 days of incubation. Results are shown as the percentage of G418-resistant total CFC. Results are the mean ± 1 SEM, and n indicates the number of experiments.

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**Table 2. PCR Analysis of Colonies From Reconstituted Mice**

<table>
<thead>
<tr>
<th>Retroviral Vectors</th>
<th>BM Colonies/PDGF B R+ Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCV</td>
<td>20/31 (65%)</td>
</tr>
<tr>
<td>MGDF-MSCV</td>
<td>35/48 (73%)</td>
</tr>
</tbody>
</table>

Individual granulocyte/macrophage colonies were picked from day 8-9 methylcellulose cultures and analyzed by PCR with neo-specific and PDGF B receptor-specific primers. Results were from 5 mice killed at 7 weeks posttransplantation. The neo-positive colonies in the spleen and the PB of MSCV mice were not assessed because of low frequency of colony formation.
23-fold increase of CFU-E, BFU-E, GM-CFC, CFU multi, and MK-CFC, respectively, compared to control mice. The mononuclear cells (MNC) in PB of control mice contained barely detectable levels of progenitors. MGDF overexpressing mice contained greatly increased numbers of all types of progenitor cells in the PB (Table 3). Progenitor cell analysis of mice killed at 10 weeks postransplantation showed similar results (data not shown). These data demonstrated that MGDF overexpressing mice had reduced hematopoiesis in the BM, especially depletion of CFU-E, but these animals had extensive extramedullary hematopoiesis in the spleen and greatly increased circulating progenitors in the PB.

### Histopathology of MGDF Overexpressing Mice

Apart from animals killed for histologic analysis, all the mice from both the MGDF overexpressing group and the control group were alive and healthy with no obvious physical changes up to more than 4 months postransplantation.

On analysis, the MGDF overexpressing mice had enlarged spleens (2 to 3 times by weight and onefold to threefold by volume) compared to control mice. However, no difference was observed in total nucleated cells recovered per spleen. As shown in the photomicrographs in Fig 5, the spleens from the MGDF overexpressing group had marked numbers of megakaryocytes that frequently exhibited endomitotic activity. Cytologically, the megakaryocytes were quite heterogeneous in appearance (Fig 5B). Most of the cells could be conventionally classified as type II cells; however, the nuclei of these cells were markedly pleomorphic, hyperlobulated and enlarged, and the amount of associated cytoplasm was variable. Naked megakaryocyte nuclei, often with degenerative features, were commonly found. The splenic megakaryocytes in both the control and MGDF overexpressing groups stained positively for vWF (Fig 5C and D). In addition to verifying the marked megakaryocyte increase in MGDF overexpressing mice, the vWF immunohistochemistry showed a marked increase of platelets in the red pulp. The spleen in the MGDF overexpressing group also contained foci of both erythropoiesis and myelopoiesis (Fig 5B). In the livers of the mice from the MGDF overexpressing group, numerous megakaryocytes and foci of erythropoiesis and myelopoiesis were also found (data not shown). Occasional islands of megakaryopoiesis were also observed in the sinuses of lymph nodes from the MGDF mice (data not shown).

In all of the other tissues in MGDF overexpressing animals, intravascular platelets were markedly elevated. Importantly, no evidence of ischemic damage to any of the organs was observed. In the lung, in addition to increased number of platelets, large irregularly shaped hematoxyphilic structures, morphologically consistent with megakaryocyte nuclei, were frequently observed in the alveolar capillary lumena (data not shown).

The BM from MGDF overexpressing mice were quite pale on harvest, likely because of decreased erythropoiesis. BM harvests became more difficult at 10 weeks postransplantation because needles used to ream the marrow space could not be introduced into the medullary space, perhaps because of the presence of fibrous or other solid tissue. Femoral radiographs of MGDF overexpressing animals (Fig 6B) showed a blurring of the usually distinct margin (Fig 6A) that exists between the diaphyseal cortical bone and medullary compartment. Additionally, the femurs appeared more radiodense in the MGDF overexpressing animals. Histologic examination of the femurs from the MGDF overexpressing mice 10 weeks postransplantation showed that the normal marrow elements had been almost completely replaced by a combination of new bone trabeculae and a fibrotic marrow containing numerous megakaryocytes (Fig 6C through F). The new bone trabeculae merged with the cortical bone, thereby obscuring the usually clear demarcation of the endosteal surface of the cortical bone from the marrow cavity (Fig 6D). The peristeum was not impacted in the MGDF overexpressing mice. At higher magnification, the nonossous marrow from the MGDF overexpressing mice appeared fibrotic with diminished levels of both erythropoiesis and myelopoiesis. In contrast, megakaryopoiesis was prominent (see inset, Fig 6F). The nonossous connective tissue in the marrow from the MGDF overexpressing mice did not stain well with the Masson's trichrome stain (data not shown). However, reticulin stains showed a marked level of reticulin deposition in the marrow space of the MGDF overexpressing mice (Fig 6H). In areas where new bone trabeculae were found, the bone matrix was almost entirely surfaced by large active osteoblasts (data not shown). In various other foci, cement lines, irregular trabecular surface contours, and multinucleated, tartrate-resistant acid phosphatase positive cells consistent with osteoclasts could be found, suggesting that the new bony trabeculae were being focally resorbed and replaced. In support of this contention, polarization microscopy of the bone (data not shown) formed in the MGDF.
overexpressing mice demonstrated both woven and lamellar matrix, consistent with a bone-forming process involving both de novo bone synthesis and remodeling.

Transplantation of Spleen Cells From MGDF Overexpressing Mice Into Secondary Recipients

Spleen cells (1.5 × 10^7) from 10 MGDF overexpressing mice, killed at 7 or 10 weeks posttransplantation, were transplanted into 25 secondary lethally irradiated mice. BM cells (5 × 10^6) from control mice were also transplanted into secondary recipients (Fig 7). Transplantation of the same number of spleen cells from control mice to secondary lethally irradiated mice resulted in only 30% survival at 3 weeks (data not shown). All mice transplanted with spleen
Table 3. Hematopoietic Precursor Analysis of Mice Reconstituted With BM Cells Infected Either With Control Vector (MSCV) or With MGDF-MSCV Vector (MGDF)

<table>
<thead>
<tr>
<th>Items</th>
<th>MSCV (n = 5)</th>
<th>MGDF (n = 5)</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell no. $\times 10^3$/f</td>
<td>15.1 ± 0.7</td>
<td>3.3 ± 0.6</td>
<td>-4.6</td>
</tr>
<tr>
<td>CFU-E/F</td>
<td>22,441 ± 784</td>
<td>135 ± 61</td>
<td>-166</td>
</tr>
<tr>
<td>BFU-E/F</td>
<td>7,407 ± 835</td>
<td>2,451 ± 539</td>
<td>-3.0</td>
</tr>
<tr>
<td>CFCmulti/F</td>
<td>3,920 ± 277</td>
<td>1,493 ± 421</td>
<td>-2.6</td>
</tr>
<tr>
<td>GMF</td>
<td>30,790 ± 1,335</td>
<td>13,403 ± 2,972</td>
<td>-2.3</td>
</tr>
<tr>
<td>MK-CFC/F</td>
<td>1,284 ± 280</td>
<td>3,933 ± 890</td>
<td>+3.3</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell no. $\times 10^3$/spl</td>
<td>17.3 ± 1.1</td>
<td>17.5 ± 1.9</td>
<td>+1.0</td>
</tr>
<tr>
<td>CFU-E/spl</td>
<td>45,200 ± 7,500</td>
<td>257,000 ± 56,000</td>
<td>+5.7</td>
</tr>
<tr>
<td>BFU-E/spl</td>
<td>12,500 ± 1,800</td>
<td>133,000 ± 11,400</td>
<td>+11</td>
</tr>
<tr>
<td>CFCmulti/spl</td>
<td>630 ± 290</td>
<td>17,600 ± 2,400</td>
<td>+28</td>
</tr>
<tr>
<td>GM/spl</td>
<td>5,600 ± 500</td>
<td>222,000 ± 32,500</td>
<td>+40</td>
</tr>
<tr>
<td>MK-CFC/spl</td>
<td>2,700 ± 500</td>
<td>61,000 ± 10,200</td>
<td>+23</td>
</tr>
<tr>
<td>PB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNC $\times 10^6$/mL</td>
<td>2,451 ± 835</td>
<td>7,407 ± 539</td>
<td>+3.3</td>
</tr>
<tr>
<td>BFU-E/mL</td>
<td>10 ± 5</td>
<td>3,500 ± 1,800</td>
<td>+350</td>
</tr>
<tr>
<td>CFCmulti/mL</td>
<td>3 ± 2</td>
<td>560 ± 150</td>
<td>+187</td>
</tr>
<tr>
<td>GM/mL</td>
<td>31 ± 12</td>
<td>9,370 ± 1,600</td>
<td>+302</td>
</tr>
<tr>
<td>MK-CFC/mL</td>
<td>12 ± 7</td>
<td>2,770 ± 570</td>
<td>+231</td>
</tr>
</tbody>
</table>

Mice were analyzed at 7 weeks posttransplantation. Table shows the number of precursors in the BM, spleen, and PB. Results are mean ±1 SEM and indicate the numbers of colonies per femur, per spleen, and per milliliter of blood, respectively. "-" or "+" indicates a decrease or an increase, respectively. P value was obtained by t-test. n, number of mice.

Cells from MGDF overexpressing mice were alive at 18 weeks. Platelet levels in these animals increased with slower kinetics compared with the primary recipients and plateaued at approximately $3,500 \times 10^6$/mL. No differences were observed in RBC and WBC counts or other hematopoietic lineages. These results showed sustained overexpression of MGDF for over 18 weeks in the primary and secondary reconstituted animals by retroviral-mediated gene transfer and the specificity of the MGDF effect for the megakaryocyte lineage.

DISCUSSION

In this report we have evaluated the effect of chronic exposure to MGDF in mice. Similar methodologies have been used to study other hematopoietic growth factors. Unlike the fatal disease and tissue damage occurring in mice with excess levels of GM-CSF or IL-3, chronic elevations of MGDF levels did not lead to premature death in animals up to the 4 months of evaluation reported here. The primary histology noted in the tissues of MGDF overexpressing mice was the marked stimulation of megakaryopoiesis with high numbers of megakaryocytes in the BM, spleen (contributing to organ enlargement), liver, and lymph nodes. Consistent with the increased megakaryocyte numbers, platelet levels were increased fivefold in the blood. This elevation persisted for 16 weeks or longer. The rate of recovery of platelet levels

![Fig 5. MGDF overexpressing mice exhibit marked splenic megakaryopoiesis. Histologic sections taken from the spleens of control (A and C) and MGDF overexpressing (B and D) mice were stained with hematoxylin and eosin (A and B) or immunostained (C and D) using antibodies directed against vWF. Photomicrographs were taken using a 20x objective.](image-url)
MGDF overexpressing mice exhibit myelofibrosis and osteosclerosis. (A and B) Radiographs of femurs from control and MGDF overexpressing mice. The small arrows denote the cortical bone of the diaphysis. (C through H) Photomicrographs of sections of femurs from control (C, E, and G) or MGDF overexpressing (D, F, and H) mice prepared using hematoxylin and eosin (C and D), antibodies (E and F) directed against vWF, or Gomori's reticulum stains (G and H). In (C) and (D), the asterisks denote cortical bone in the metaphysis. The arrowheads in (C) show the clear demarcation between the cortical bone and medullary marrow space in control animals, which is not present in MGDF overexpressing mice. In (G), the arrowhead denotes a bone trabeculum, and the arrowhead in (H) is directed at a typical argyrophilic reticulum fiber. The photomicrographs were taken with the 4x objective in (C) through (F), except for the insets in (E) and (F), which used a 40x objective. The photomicrographs of the reticulum stains (G and H) were prepared using the 40x objective.

After transplantation is consistent with unpublished data we have generated using daily injection of human MGDF in irradiated and transplanted mice. Mice injected daily with MGDF reached preirradiation levels 6 to 8 days faster than carrier controls. A similar effect was seen in this study, demonstrating that the MGDF overexpressing mice represent a valid model of chronic MGDF administration.

Our study also raised another interesting issue that remains to be addressed: ie, the regulation of platelets by MGDF. After transplantation, platelet number in MGDF overexpressing mice increased after 1 week and reached a plateau at 4 to 5 weeks and maintained these high levels (Fig 4A). MGDF activity in plasma was the highest at 1 week but decreased sharply and was no longer detected at 4 weeks. However, conditioned media from spleen cells of MGDF overexpressing mice killed at 10 weeks clearly showed that MGDF was still being produced but undetected in circulation. The most likely hypothesis is that high levels of platelets bind circulating MGDF. Alternatively, soluble mpl receptor in the circulation may also play a role in binding MGDF.

Intravascular platelets were also noted in other organs including the lung (data not shown). Whether the platelets were trapped or shed from circulating megakaryocytes remains to be determined. The only significant effect noted on other hematopoietic lineages was a decrease in RBC counts associated with decreased hemoglobin and hematocrit levels.

Whether this a direct effect of MGDF or a secondary mecha-
nism is unknown. Increased numbers of hematopoietic precursors in spleens and periphery in MGDF overexpressing mice are most likely a result of reduced hematopoiesis in the BM but a shift to extramedullary sites.

Interestingly, the BM of MGDF overexpressing mice showed myelofibrosis and osteosclerosis confined to the medullary cavity. Osteoclasts and evidence of bone remodeling were present, indicating that osteoclast formation from hematopoietic precursors was not completely abrogated in these mice. MGDF could potentially target nonhematopoietic cells, such as endothelial cells, which subsequently release bone and stromal cell growth factors locally. However, it is most likely that the myelofibrosis and osteosclerosis is an indirect effect due to the marked expansion of megakaryocytes because c-mpl, the receptor for MGDF, is most highly expressed on hematopoietic cells.4,22 No evidence for fibrosis is seen in nonhematopoietic, nonmegakaryocyte-containing, but vessel-rich, organs such as the kidney in the MGDF overexpressing animals. Clinically, myelofibrosis has been associated with increased narrow megakaryocytes and it has been postulated that growth factors released from megakaryocytes, such as PDGF and/or transforming growth factor-β (TGF-β), may be responsible for the development of the myelofibrosis.25-28 It is possible that these factors stimulate stromal cells and osteoblasts. PDGF-BB, in particular, appears to stimulate endosteal bone formation and a “myelofibrosis-like” change in the BM of rats (D. Lacey, Amgen Inc, unpublished data, August 1994). TGF-β also simulates new bone formation when administered in vivo.29 Furthermore, both PDGF and TGF-β have been suggested as etiologic factors in idiopathic myelofibrosis.30,31 Because megakaryocytes synthesize a wide range of mediators that could target the skeleton, other factors could, of course, be involved. These animals may offer a model for myelofibrosis and/or osteosclerosis to study the basis of the disease and potential therapeutic treatment.

Although the underlying cause of the myelofibrosis and osteosclerosis seen in the MGDF overexpressing mice is unknown, it is unlikely that any similar effects will be seen clinically in humans. Most uses of MGDF will probably involve short-term injections of low doses to overcome severe thrombocytopenia. In addition, megakaryocyte levels in the BM will be low after chemotherapy. Even though mice injected with MGDF develop myelofibrosis, this condition recedes once the factor is withdrawn (Del Castillo and Ulich, Amgen Inc, unpublished data, March 1995). Furthermore, rhesus monkeys given doses of MGDF resulting in an approximately 8- to 10-fold elevation of platelet levels over a 28-day treatment did not show any fibrotic effects in the BM (T. Kirley, Amgen Inc, unpublished data, March 1995) and when normal, nonhuman primates are administered up to 100× the maximally effective dose of MGDF per day for 28 days, no evidence of BM fibrosis was observed.32 Therefore, the present data suggest that MGDF is a primary stimulator of megakaryopoiesis in vivo with limited effect on other hematopoietic lineages. The data also suggest that carefully controlled administration of MGDF might be useful in the clinical management of patients recovering from thrombocytopenia induced by cytotoxic therapies (radiotherapy or chemotherapy). That MGDF had no effect on neutrophilic recovery further suggests that MGDF and G-CSF may potentially be combined to provide for rapid recovery of both platelets and neutrophils.

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