Analysis of Hematopoiesis in max 41 Transgenic Mice That Exhibit Sustained Elevations of Blood Granulocytes and Monocytes

By Donald Metcalf, Geoffrey J. Lindeman, and Nicos A. Nicola

An unusual mouse line (max 41) that carries an inserted transgene encoding the nuclear transcription factor, Max, exhibits a 50- to 60-fold elevation of blood neutrophils and a 10-fold elevation of blood monocytes. Analysis showed that these elevated levels of blood cells were sustained incrementally by a sevenfold increase in mature neutrophils to stimulation in vitro by the four colony-stimulating factors. The study has documented that both granulocytes and monocytes are generated in excess numbers in max 41 mice by progressive size increments in precursor populations and have highlighted the previously ignored situation that most neutrophils generated in the marrow probably never leave the organ.

Materials and Methods

Mice. The generation of the max 41 transgenic line has been described elsewhere.1 The mice are (C57BL × SJL)F1, mice and, because the phenotype is dominant in heterozygous mice, the line was maintained by crossing transgenic mice with normal (C57BL × SJL)F1, mice held under specific pathogen-free conditions. Transgenic mice were identified by polymerase chain reaction on blood leukocyte DNA using oligonucleotides specific for the transgene. Observations were performed on max 41 transgenic mice of either sex aged 6 to 8 weeks and on control mice from the same litters. Mice were anesthetized and white cell, platelet, hematocrit, and erythrocyte sedimentation estimates performed on the orbital plexus blood. The mice were then reanesthetized and exsanguinated via the auricular vessels. A volume of 2 mL of 0.9% saline containing 5% newborn calf serum was injected intraperitoneally and, after gentle abdominal massage, the peritoneal cell suspension was harvested using a soft plastic pipette. The spleen was weighed and the marrow collected from one femur. Dispersed cell suspensions were prepared from spleen and bone marrow tissue and cytocoentrifuge preparations made, then stained with May-Grünwald-Giemsa. The remaining organs were fixed in 10% formalin for histologic examination. Fetal livers were obtained at day 14 of pregnancy and dispersed cell suspensions prepared by pipetting.

Culture studies. Cultures were performed using 1-mL volumes of agar-medium in 35-mm plastic Petri dishes.2 The numbers of cells per milliliter routinely cultured were 25,000 BM cells, 50,000 spleen or peritoneal cells, and 10,000 fetal liver cells. The medium used was Dulbecco’s modified Eagle’s medium containing a final concentration of 20% newborn calf serum and 0.3% agar (Bacto agar, Difco, Detroit, MI). Colony formation was stimulated by inclusion in the cultures of 0.1 mL of 0.9% saline and 5% (vol/vol) fetal calf serum containing 10 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, M-CSF or Multi-CSF (interleukin-3 [IL-3]), 100 ng rat stem cell factor (SCF) or 500 ng IL-6. All stimuli were recombinant factors purified to homogeneity after production using either bacterial or yeast expression systems (50 U/mL is defined as the concentration stimulating half-maximal numbers of colonies to develop in 1-mL cultures of 75,000 C57BL BM cells and the specific activities of the CSFs used were 10 U/mg for G-CSF, M-CSF and Multi-CSF or 3 × 10^4 U/mg for GM-CSF).

The cell suspensions in agar-medium were added to the culture dishes, and, after thorough mixing with the stimulus, the cultures were allowed to gel, then incubated for 7 days at 37°C in a fully humidified atmosphere of 10% CO2 in air. After incubation, colony formation (clones of more than 50 cells) was scored at × 35 magnifications using an Olympus dissection microscope (Olympus Optical Co, Tokyo, Japan). The cultures were then fixed by adding 1 mL of 2.5% glutaraldehyde. After fixation for 4 hours, the intact cultures were floated onto glass slides, allowed for 20 min at 4°C, and stained with methylene blue. Colony numbers were obtained by counting the colonies using a dissecting microscope at × 35 magnification

From The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

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Address reprint requests to Donald Metcalf, MD, The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, 3050 Victoria, Australia.

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HEMATOPOIESIS IN max 41 TRANSGENIC MICE

Table 1. Blood Cell Counts in max 41 Versus Littermate Control Mice

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Mice</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Platelets</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>max 41</td>
<td>143</td>
<td>25,470 ± 21,710</td>
<td>5,820 ± 2,780</td>
<td>3,130 ± 2,940</td>
<td>40 ± 150</td>
<td>731,000 ± 147,000</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Littermate</td>
<td>143</td>
<td>540 ± 400</td>
<td>5,000 ± 2,230</td>
<td>270 ± 170</td>
<td>70 ± 80</td>
<td>928,000 ± 125,000</td>
<td>46 ± 3</td>
</tr>
</tbody>
</table>

Mean values ± SD.

RESULTS

Adult 6- to 8-week-old transgenic max 41 mice appeared in good health and were indistinguishable from littermate control mice. However, as shown in Table 1, they exhibited a 50-fold elevation of neutrophils in the PB and a 10-fold elevation of monocytes with normal levels of lymphocytes and eosinophils. Hematocrit values were normal, but the max 41 mice exhibited slightly reduced platelet levels. Most neutrophils and monocytes exhibited a normal morphology, but a small percentage appeared slightly immature in morphology with incomplete nuclear lobulation of the neutrophils and slightly immature and basophilic cytoplasm of the monocytes.

At autopsy, several abnormalities were seen consistently in max 41 mice. There was a twofold to fourfold increase in spleen size, the spleen being pinker in color than normal and without visible lymphoid follicles. The lymph nodes tended to be slightly enlarged and had a yellowish-green color, and the BM lacked the usual red color. Peyer's patches tended to be small or absent.

On histologic examination, the BM in max 41 mice was tightly packed by cells with an obvious dominance of granulocytic cells. The spleen exhibited gross depletion of lymphoid follicles with expansion of the red pulp by hematopoietic cells again dominated by granulocytic cells, although erythroid and megakaryocytic cells were also evident. The liver showed a varying degree of portal infiltration by hematopoietic cells almost exclusively composed of granulocytic cells, and the lymph nodes showed enlargement of the hilar regions by infiltrating granulocytic cells associated with a reduced cellularity of lymphoid regions. The thymus exhibited no obvious histologic abnormality, and no cellular infiltrates were observed in the kidney, heart, muscle, or other nonhematopoietic organs with the exception of the lung, which showed a variable degree of peribronchial granulocyte cuffing and/or infiltration of mature granulocytes in the alveolar walls.

Cytology of hematopoietic organs. As shown in Table 2, total femur cell counts in max 41 mice were slightly increased above those in control mice and differed consistently in composition. Max 41 populations exhibited a threefold elevation in the percentage of mature neutrophils and a significant increase in immature granulocytes. The elevated proportion of granulocytic cells in the max 41 marrow was
Table 3. Progenitor Cell Content of the BM in max 41 and Littermate Mice

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Total Colonies</th>
<th>G</th>
<th>GM</th>
<th>M</th>
<th>Eo</th>
<th>Meg</th>
<th>Blast</th>
</tr>
</thead>
<tbody>
<tr>
<td>max 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>136 ± 12</td>
<td>31 ± 16</td>
<td>27 ± 5</td>
<td>76 ± 14</td>
<td>1 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>51 ± 23</td>
<td>45 ± 23</td>
<td>3 ± 1</td>
<td>2 ± 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M-CSF</td>
<td>131 ± 40</td>
<td>34 ± 10</td>
<td>51 ± 23</td>
<td>45 ± 25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multi-CSF</td>
<td>128 ± 15</td>
<td>48 ± 18</td>
<td>28 ± 7</td>
<td>43 ± 34</td>
<td>1 ± 1</td>
<td>3 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>SCF</td>
<td>101 ± 19</td>
<td>84 ± 23</td>
<td>5 ± 7</td>
<td>2 ± 3</td>
<td>0</td>
<td>0</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>Littermate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>59 ± 15</td>
<td>22 ± 7</td>
<td>10 ± 3</td>
<td>24 ± 8</td>
<td>3 ± 1</td>
<td>0.3 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>17 ± 5</td>
<td>16 ± 6</td>
<td>1 ± 1</td>
<td>0.2 ± 0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M-CSF</td>
<td>79 ± 45</td>
<td>5 ± 4</td>
<td>7 ± 4</td>
<td>67 ± 38</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multi-CSF</td>
<td>61 ± 28</td>
<td>23 ± 11</td>
<td>10 ± 3</td>
<td>13 ± 8</td>
<td>2 ± 1</td>
<td>8 ± 5</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>SCF</td>
<td>28 ± 13</td>
<td>20 ± 8</td>
<td>1 ± 1</td>
<td>1 ± 2</td>
<td>0</td>
<td>0</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

All cultures contained 25,000 BM cells from mice aged 6 to 8 weeks. Colony formation was scored after 7 days of incubation and the data are the mean values ± SD from representative cultures from six mice per group.

Table 4. Progenitor Cell Content in the Spleen of max 41 Transgenic and Littermate Control Mice

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Total Colonies</th>
<th>G</th>
<th>GM</th>
<th>M</th>
<th>Eo</th>
<th>Meg</th>
<th>Blast</th>
</tr>
</thead>
<tbody>
<tr>
<td>max 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>79 ± 16</td>
<td>16 ± 16</td>
<td>46 ± 1</td>
<td>1 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>26 ± 25</td>
<td>1 ± 0</td>
<td>7 ± 1</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M-CSF</td>
<td>59 ± 26</td>
<td>14 ± 19</td>
<td>19 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multi-CSF</td>
<td>74 ± 21</td>
<td>16 ± 21</td>
<td>19 ± 1</td>
<td>1 ± 9</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>IL-6</td>
<td>42 ± 32</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Littermate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
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</tr>
<tr>
<td>M-CSF</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multi-CSF</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-6</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Cultures contained 50,000 spleen cells and were stimulated by 10^3 U/mL of GM-CSF, G-CSF, M-CSF, or Multi-CSF or 500 ng IL-6. Colonies were scored after 7 days of incubation. The frequencies of different colony types were determined from the stained cultures. Data shown are mean values from duplicate cultures.

Associated with a greatly reduced frequency of lymphocytes and nucleated erythroid cells.

The enlarged spleen of max 41 mice characteristically exhibited a 20-fold increase in the percentage of mature and immature granulocytes and a marked depletion of lymphoid cells. It was notable that the ratio of immature to mature granulocytes in the peritoneal cell population of those committed to granulocyte or granulocyte-macrophage formation was always higher than that in the marrow. These changes in spleen populations were accompanied by a twofold to threefold increase in the percentage of immature granulocytes and a marked depletion of lymphoid cells.

No change was observed in the total number of peritoneal cells in max 41 mice, but there was a consistent depletion in the percentage of lymphocytes and a higher than normal percentage of macrophages, the latter cells being normal in morphology. Small numbers of mature neutrophils were consistently observed in the peritoneal cell population of max 41 mice. No elevation of mitotic activity was observed in the peritoneal cells of max 41 mice.

Progenitor cell levels. As shown in Table 3, in cultures of max 41 BM cells stimulated either by G-CSF, GM-CSF, M-CSF, Multi-CSF, or SCF, the frequency of progenitor cells was two to three times higher than in littermate control cultures. Analysis of the absolute numbers of various progenitor cells showed that the most consistently elevated were those committed to granulocytic or granulocyte-macrophage formation. In contrast, the frequencies of eosinophil, megakaryocyte, and blast colony progenitors were not elevated in max 41 marrow cultures. These general findings were supported by data from additional cultures stimulated by IL-6, or SCF plus G-CSF. A curious and quite consistent feature of max 41 cultures stimulated by M-CSF was the unusually high frequency of granulocytic and granulocyte-macrophage colonies in contrast to the pattern of M-CSF-stimulated cultures as seen in littermate cultures where, as expected from the usual behavior of M-CSF, macrophage colonies were the dominant type. Cultures of max 41 marrow containing varying concentrations of M-CSF from 10 to 1,000 U/mL also exhibited a high frequency of granulocyte-containing colonies (data not shown).

In cultures of spleen cells, a somewhat different pattern was observed. The overall frequency of progenitor cells was 50-fold higher in max 41 cultures than in control cultures (Table 4). Although granulocyte-containing colonies were again the dominant colony type in max 41 cultures, major increases were also observed in macrophage, eosinophil, megakaryocyte, and blast progenitor cells. As was noted in the marrow cultures, max 41 spleen cells also showed the distinctive feature, when stimulated by M-CSF, of producing a high proportion of granulocytic and granulocyte-macrophage colonies.

In cultures of peritoneal cells, no differences were noted between max 41 and control cultures and in both types of culture only occasional colonies developed.

The colonies developing in stimulated cultures of max 41 BM or spleen cells were similar in size and morphology to those produced by littermate cells and contained comparable populations of cells at varying stages of differentiation. A comparison of the quantitative responsiveness of the progenitor cells in max 41 and control marrow populations to stimu-
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The blast colonies chosen for analysis were those stimulated by SCF plus G-CSF, a combination shown from previous studies to enhance the size of blast colonies, but not to alter the number of blast colonies forming or the relative frequency of different types of progenitor cell present in the blast colonies.3

After 7 days of stimulation by SCF plus G-CSF, individual blast colonies (23 from max 41 cultures, 25 from littermate control cultures) were removed, the colony cells resuspended in agar-medium, and recultured in duplicate colonies stimulated by spleen conditioned medium (SCM) containing a mixture of growth factors or by G-CSF or M-CSF. In SCM-stimulated secondary cultures, no significant differences were noted between the two types of blast colony in the absolute numbers (131 ± 142 for max 41 colonies, 220 ± 227 for littermate control colonies) or types of progenitor cells present in the colonies. In both cases, most progenitor cells were committed to the macrophage lineage and max 41 colonies did not contain a preponderance of granulocyte-committed progenitor cells. Similarly, no significant differences were noted in the frequency of granulocyte progenitor cells detectable using G-CSF and, indeed, somewhat lower numbers of such progenitors were present in max 41 colonies than in control colonies. A notable feature of the secondary cultures stimulated by M-CSF was that, from both types of blast colony, the dominant colony type developing was composed of macrophages (97% for max 41 colonies, 94% for littermate control colonies), the usual pattern in M-CSF-stimulated cultures. This was in striking contrast to the anomalous behavior in cultures of unfractionated max 41 marrow, spleen, or fetal liver cells, which when stimulated by M-CSF, developed a high frequency of granulocyte and granulocyte-macrophage colonies. In these secondary cultures, no differences in colony size or morphology were observed between the colonies derived from the two types of blast colony.

Kinetics of granulocyte and monocyte populations in max 41 mice. To investigate the possibility that the abnormally high levels of granulocytes and monocytes in the PB might be caused by abnormally long lifespans of the cells in the PB or to premature release of mature cells from the marrow, kinetic studies were undertaken, after tritiated thymidine labeling, of groups of 6- to 8-week-old max 41 transgenic and littermate control mice.

As shown in Fig 2, no differences were observed in the rate of accumulation of labeled mature neutrophils in the marrow between max 41 and control mice. In both cases, the entire population was replaced by new (labeled) cells over a 72-hour period. A similar lack of difference was noted in the accumulation of labeled monocytes in the marrow (data not shown).

Analysis of the accumulation of labeled neutrophils and monocytes in the PB (Fig 3) also failed to show any significant differences between max 41 and control mice. In both types of mice, no labeled neutrophils appeared in the PB before 24 hours and, by 72 hours, most cells were labeled. Similarly, in both types of mice, labeled monocytes began to appear at 6 hours and approximately 80% of monocytes were labeled by 72 hours.
The data indicate that the turnover times of neutrophils and monocytes in the marrow and PB are essentially identical in max41 transgenic and control mice.

**DISCUSSION**

Young adult max41 transgenic mice exhibit a selective increase in granulopoiesis and monocyte formation that does not have the pathologic features of myeloid leukemia, although it does result in a dominant population of granulocytic cells in the marrow, in spleen enlargement mainly caused by granulocytic cells, and abnormal granulocyte infiltration in the liver, lymph nodes, and lung. Associated with these hematopoietic changes is a major depletion in the numbers of B lymphocytes in the marrow, spleen, lymph nodes, and peritoneal cavity. On the basis of these changes, one proposal has been that a fundamental abnormality in differentiation commitment may exist in max41 precursor hematopoietic cells, leading to inadequate commitment to the B-lymphoid lineage and excess commitment to the granulocyte-macrophage lineage. The present study has not sought to obtain evidence of this possibility, but has merely sought to establish the cellular basis permitting a max41 mouse to exhibit sustained excess blood levels of granulocytes and monocytes.

The situation shown in young adult max41 mice can be summarized in Fig 4, which presents in an accurate scale drawing the total size of the various granulocytic cell compartments. For this purpose, only the total marrow and spleen cell numbers have been calculated and the populations in the liver and lymph nodes (which, on analysis, comprised approximately an additional 15%) have been excluded. The data show that a sevenfold to eightfold increase in mature granulocytes in the marrow plus spleen is impressive, it is in striking contrast to the 50- to 60-fold increase in blood neutrophils in the max41 mice. To understand why this difference should exist, the relative sizes of the marrow plus spleen versus the blood population need to be considered in both normal and max41 mice (Fig 4), as do the turnover times of mature cells in the two populations. The fate of blood neutrophils also needs to be considered, although information on this general question is very incomplete in the literature. It is generally agreed that an intravascular pool of margined neutrophils exists that, at least in humans, approximates the numbers of circulating neutrophils. It is also evident that neutrophils exit to the tissues, saliva, and gut in a one-way direction. However, the numbers involved are not known. These events for neutrophils in normal lit-
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LITTERMATE

Progenitor Cells \rightarrow Immature Granulocytes \rightarrow Mature Granulocytes

Blood

Progenitor Cells \rightarrow Immature Granulocytes \rightarrow Mature Granulocytes

max41

X4 \quad X8 \quad X7 \quad X60

Blood

Fig 4. Scale drawings of the total numbers of various types present in the marrow plus spleen compared with total mature granulocytes present in the blood of littermate and max 41 mice.

terminer control mice are shown drawn to scale in Fig 5, with the possible size of the extravascular pool estimated from the known half-life of neutrophils in the circulation (8 hours), and the observation that the whole blood neutrophil population was replaced by marrow-derived cells every 48 hours. This suggests that the tissue pool might be approximately three times the size of the blood pool, but to be very conservative, the drawing indicates a pool size 14 times that of the blood pool. It is evident by comparing the size of the marrow/spleen pool (renewed every 72 hours) with that of the blood/tissue pool (renewed every 48 hours) that most neutrophils produced in the marrow do not leave the organ under basal conditions. In the past, this has been recognized, but the excess marrow neutrophils have been termed a reserve pool without spelling out the logical consequence that, unless some emergency occurs, the reserve pool must, in fact, die in situ. This situation may seem improbable, but has been well documented as occurring with T lymphocytes produced in the thymus.

As judged from the relative sizes of the marrow/spleen and blood neutrophil pools in Fig 4, max 41 mice appear to differ from normal mice in that a somewhat higher proportion of neutrophils would appear to exit from the marrow/spleen than appears to be the case with normal mice.

The final point regarding the kinetics of neutrophil production in max 41 mice that is worthy of comment relates to the major disparity between the sevenfold increase in marrow neutrophils versus the 50-fold increase in blood neutrophils. This same phenomenon is also seen in normal mice in response to the injection of G-CSF, where marrow neutrophils typically increase twofold to threefold, but blood neutrophils increase 20-fold. The converse disparity is seen in mice with inactivation of the G-CSF gene where marrow neutrophils decrease only twofold, while blood neutrophils decrease 10-fold. It seems likely that this fivefold to 10-fold difference in increases of neutrophils between marrow and blood is the consequence of the larger cell numbers in the marrow compared with the blood. With a one-way flow system and a fixed rate of cell exit from the blood to the tissues, a minor perturbation in the larger (marrow/spleen) population would produce a much larger perturbation in the smaller (blood) population.

Although the excess granulopoiesis in the max 41 marrow is associated with an almost complete loss of nucleated erythroid cells from the marrow, the max 41 mice are not anemic. Normal levels of erythropoiesis appear to be sustained by a compensatory increase in nucleated erythroid cells in the spleen. Thus, despite the slight thrombocytopenia, the sustained excessive granulopoiesis in max 41 mice does not seem to result in any major overall deficiency in erythrocyte or platelet formation.

The present analysis of max 41 mice has documented two abnormalities: (1) a stepwise overproduction of granulocytes and monocytes in the marrow and spleen, and (2) an increased exit of mature granulocytes and monocytes to the blood. The latter anomaly may be based on some abnormality in the mechanisms controlling cell exit from the marrow or may be the simple mechanical consequence of overcrowding in the marrow. Supporting the latter possibility is the histologic appearance of the max 41 BM, which is not only extremely tightly packed, but sometimes exhibits herniation of marrow cells through the foramina of the bone.

This study has not addressed in any detail the possible mechanisms responsible for the increased granulocyte and monocyte formation in the max 41 mice, and studies on this question are in progress. However, the data shown do indicate that the precursor cells in max 41 mice are neither autonomous nor hyperresponsive to stimulation by the four CSFs. The only obvious abnormality noted was that stimulation by M-CSF resulted in the formation of an unusually high proportion of granulocyte-containing colonies. Although this characteristic was seen in cultures of marrow, spleen, and
fetal liver cells, its significance is unclear because recloned blast colonies gave the expected high proportion of macrophage colonies when stimulated by M-CSF, suggesting that the phenomenon may have an indirect mechanism.

Although max 41 mice exhibit many features of mice injected with large doses of G-CSF, preliminary data indicate that G-CSF levels are not elevated in the serum of max 41 mice, nor do max 41 tissues produce unusually high concentrations of G-CSF.

Recently, three other murine systems have been described in which blood or tissue granulocyte numbers are chronically elevated, transgenic mice with an inserted Kk structural gene, the histocompatibility A'b gene, and mice with inactivation of the IL-8 receptor gene. These are in need of analysis similar to that described in the present study, but these and the max 41 mice are likely to be valuable models for further studies on the consequences of long-term perturbation of granulocyte and monocyte formation, and for analyzing what abnormalities in regulatory control achieve this result.

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