Molecular Mechanisms in Myeloid Lineage Development *

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Recent work from my laboratory has described a new mechanism for controlling the expression of one hematopoietic colony-stimulating factor (CSF) receptor by the interaction of another CSF with its receptor [1]. This type of transmodulation occurs at the level of mRNA stability and has interesting implications for hematopoietic cell development. It is particularly relevant to mechanisms that determine myeloid lineage restriction and its potential involvement in this process will be presented and analyzed.

The development of eight distinct mature blood cell types occurs from a single stem cell population and represents a challenging problem in understanding the mechanisms that control both normal and abnormal growth and differentiation. As far as we now know, the development of the cells along the hematopoietic cell lineages is controlled by a set of glycoprotein factors called CSFs [2]. Some CSFs such as interleukin-3 (IL-3, also called multi-CSF) and granulocyte-macrophage CSF (GM-CSF) have the potential to stimulate development of several mature cell types, whereas macrophage CSF (M-CSF) and granulocyte CSF (G-CSF) are more restricted in their actions even though they do have some effects on early hematopoietic cell development in combinations with other CSFs [3, 4].

Our efforts to understand the mechanisms that control hematopoietic cell development have focused on the segment of the myeloid pathway that leads to mature macrophage and granulocytic cells (Fig. 1). These two cell populations arise from a common bipotential progenitor cell. The type of mature cell that develops from the bipotential progenitor depends on the concentration and type of CSF that stimulates this cell [1, 5–7]. M-CSF and G-CSF will stimulate, respectively, the formation of macrophages and granulocytes almost exclusively. IL-3 and GM-CSF stimulate formation of both macrophages and granulocytes. GM-CSF in particular exhibits an interesting preferential stimulation of macrophages at very low concentrations [1, 6, 7], but favors the formation of granulocytes at higher concentrations of the growth factor. These results indicate there are interesting effects of CSF on determining the ultimate fate of a bipotential progenitor cell; however, at present there are no indications as to how this occurs.

To study the mechanisms that influence lineage restriction we developed a cell line that expressed three of the four CSF receptors and responded to the three CSFs. The parental cell line, FDC-P1, normally has receptors for GM-CSF and IL-3 [8, 9], and a further subclone expressing M-CSF receptors was selected by forced growth with M-CSF as the sole growth factor. This cell line was called FD/MAC. Individual clones of FD/MAC cells grew with either IL-3, GM-CSF, or M-CSF as the only growth

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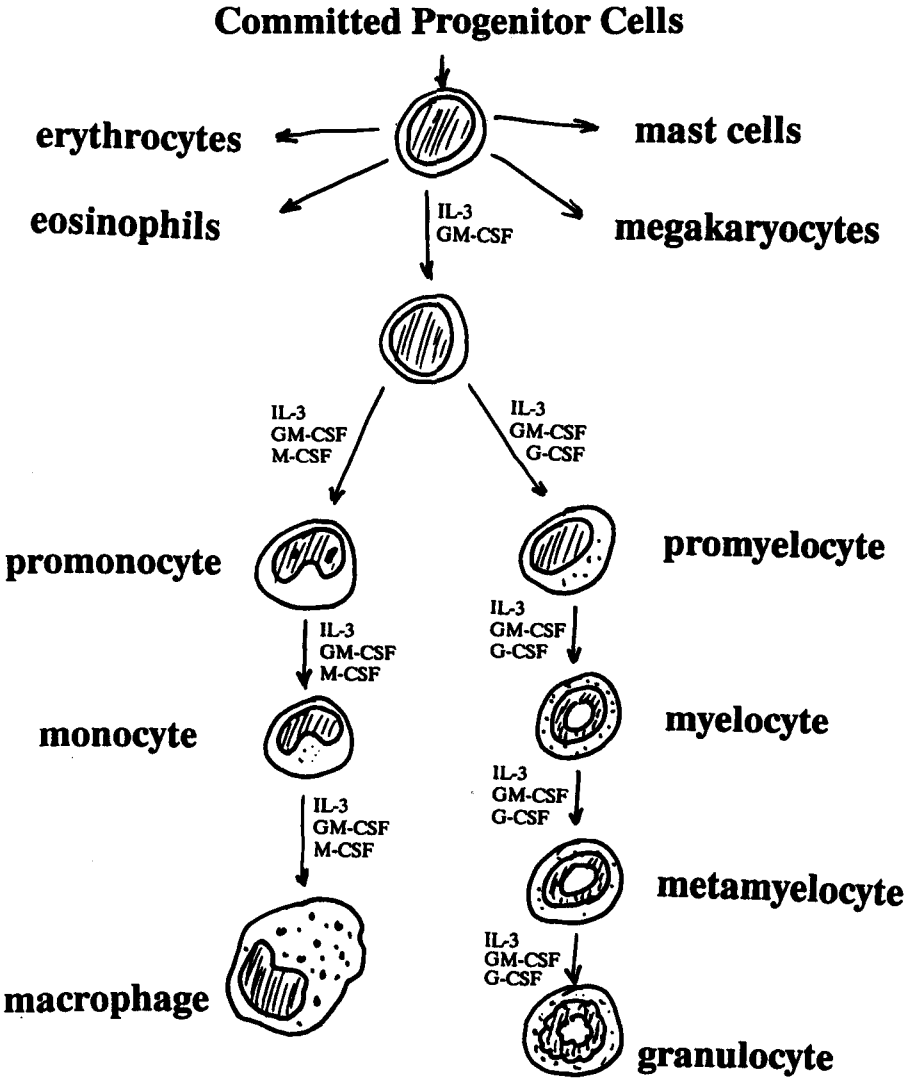

Fig. 1. Developmental scheme for the macrophage and granulocytic cell lineages. A bipotential progenitor cell can develop into either a macrophage or a granulocyte and this decision is influenced by a set of four colony stimulating factors (IL-3, GM-CSF, M-CSF, and G-CSF)

factor, and therefore each cell must express receptors for each of these CSFs. GM-CSF and IL-3 stimulated only growth in the FD/MAC cells, but M-CSF induced both growth and differentiation, as we have previously demonstrated [10].

An early observation with the FD/MAC cell line was that M-CSF receptor protein was lost after stimulation with either GM-CSF or IL-3. Factor-switching experiments demonstrated that the loss was due to the dominant actions of the stimulated GM-CSF and IL-3 receptors because removal of the FD/MAC cells from any growth factor caused expression of the M-CSF receptor (even if they had been grown in GM-CSF), while switching to growth on GM-CSF or IL-3 caused loss of the M-CSF receptor (even if they had been grown in M-CSF). The interaction of GM-CSF (or IL-3) with its receptor therefore induced some positive signal that was directly responsible for the loss of the M-CSF receptor.

The point at which this regulation occurred was found to be at the level of M-CSF receptor mRNA stability ([1], see also B. C. Gliniak, L. S. Park, and L. R. Rohrschneider, Mol Biol Cell 3, in press). Transcription, as measured by nuclear run-on assays, of the M-CSF receptor gene (c-*fms*) was not affected by the CSF in which the cells were grown, but

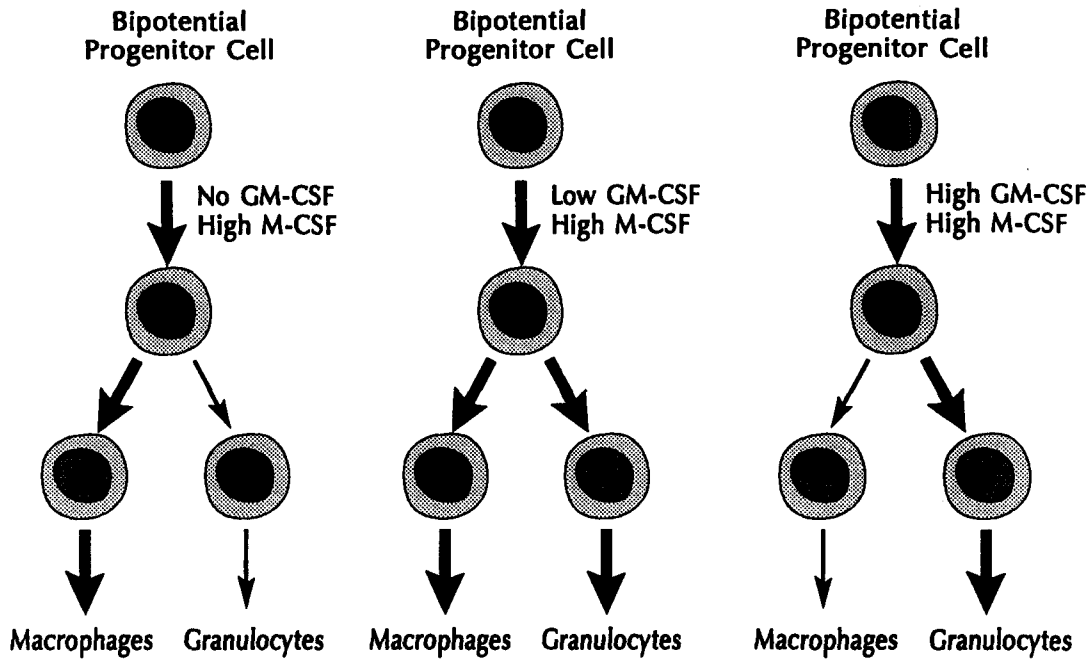

Fig. 2. Summary of the results of agar colony assays on mouse bone marrow cells grown in the presence of combinations of M-CSF and GM-CSF. The *darker arrows* indicate the favored pathways in development of a bipotential progenitor cell to mature macrophages or granulocytes grown with the growth factors indicated

Northern analyses of M-CSF receptor mRNA showed dramatic reductions (50- to 100-fold) when cells were grown in the presence of GM-CSF or IL-3. Further studies demonstrated that the reduction in M-CSF receptor mRNA caused by GM-CSF or IL-3 was dominant and concentration dependent. Low concentrations of GM-CSF had little effect on the M-CSF receptor mRNA whereas higher concentrations completely abolished M-CSF receptor mRNA. When GM-CSF was added to cells already growing in M-CSF, there was a loss of M-CSF receptor mRNA. These results indicate that the dominant effect on M-CSF receptor expression occurs posttranscriptionally.

Preliminary studies with inhibitors of transcription and protein synthesis indicate that the likely control point is right at the level determining the mRNA stability (Gliniak, Park, and Rohrschneider, Mol Biol Cell 3, in press). Experiments with actinomycin D and cyclohexamide used singly or together indicated the presence of a rapidly turning over protein (probably an RNase) that regulates the degradation of the M-CSF receptor mRNA. So far, we have no direct proof for the existence of a stabilizing factor for the M-CSF receptor mRNA reported in other cell types [11].

The above results indicate that the concentration of GM-CSF can have a dramatic influence on the expression, and therefore function, of the receptor for M-CSF. This effect was detected in a cell line and therefore, to obtain some evidence that a similar effect occurs in hematopoietic cell development, we examined the influence of combinations of M-CSF and GM-CSF on the development of murine bone marrow cells in agar assays [1]. A diagrammatic summary of the results is presented in Fig. 2. M-CSF stimulated the formation of macrophage colonies exclusively, whereas addition of even small amounts of GM-CSF caused a shift to production of granulocytic colonies and especially mixed colonies containing both granulocytes and macrophages. With increasing amounts of GM-CSF added to a fixed M-CSF concentration the shift toward more granulocytic colonies was more apparent and the

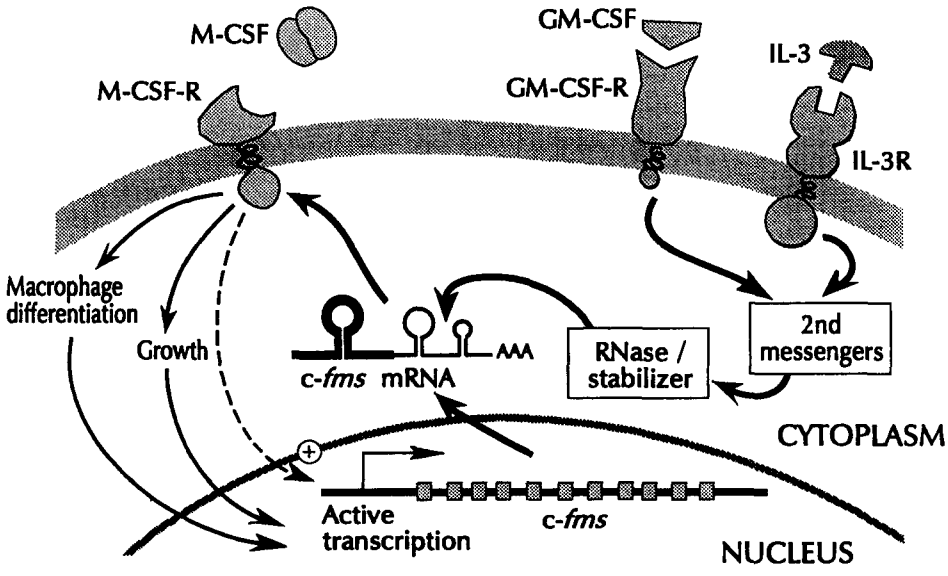

Fig. 3. Current model for how activated GM-CSF and IL-3 receptors (GM-CSF-R and IL-3R) cause destabilization of the mRNA for the M-CSF (c-*fms*) receptor (M-CSF-R). The rationale for the model is discussed in the text

mixed colonies were also numerous. The colony assays, although complex in nature because of the different cell types involved, did support the notion that GM-CSF can act in a dominant fashion to suppress development along the macrophage pathway.

The diagram in Fig. 3 illustrates our present concept for the regulation of M-CSF receptor mRNA by the interaction of GM-CSF and IL-3 with their respective receptors. The binding of M-CSF to its receptor stimulates both a differentiation and growth response, and in addition there is some evidence from the nuclear run-on studies for a small autoactivation of c-*fms* transcription [1]. GM-CSF and IL-3 also stimulate growth but send additional signals that control c-*fms* mRNA stability. Presumably some type of second messenger system is involved in sending a signal that affects c-*fms* mRNA stability. This signal ultimately can either inactivate a factor that stabilizes the mRNA or activate a factor such as an RNase that degrades the mRNA. Preliminary results suggest the latter case may be more correct, and that activation could occur through increased transcription of the factor or posttranslational modification(s). The GM-CSF (or IL-3)-induced signal for degradation of c-*fms* mRNA is dominant, and the amount of degrading activity is dependent on the GM-CSF concentration.

The dominant actions of GM-CSF on suppressing expression of the receptor for M-CSF and negatively influencing the development of mature macrophages suggests that this mechanism could help to explain lineage restriction. The decision of a bipotential progenitor cell to restrict itself to one defined lineage could be determined by the relative concentrations of growth factors encountered by that cell at the time the lineage selection is made. If the cell is in an environment rich in GM-CSF, then the M-CSF receptor will not be expressed and that cell will most likely proceed down the granulocytic pathway. If, however, the environment lacks GM-CSF then the M-CSF receptor will be expressed and that cell has the opportunity to become a macrophage. This mechanism has the advantage that a cell can respond quite rapidly to fluctuations in CSF concentration and shift lineages by controlling expression of receptors through regulation of mRNA stability. Activating or inactivating transcription machinery is not necessary.

This proposed mechanism would also suggest a slightly different purpose for

GM-CSF and its receptor. Previously, GM-CSF has been envisioned as a growth factor that merely stimulated growth and development toward mature cells. The role of GM-CSF in regulating the expression of the M-CSF receptor would now suggest an additional role of the GM-CSF receptor in determining the lineage that a bipotential progenitor cell will select. The concentration of GM-CSF may serve as a switch between alternate lineages.

Hematopoiesis may be viewed as a developmental system, and many parallels exist with other developmental model systems. The proposed role for GM-CSF in controlling lineage development by negative regulation has a counterpart in many other developmental pathways [12–18]. In these systems cell contact or signals from one developing cell inhibit development of another cell lineage, or cause that cell to select an alternate lineage. In some cases these effects are known to occur through cell surface receptors.

The new results presented here and elsewhere [1] provide a different look at hematopoiesis that may help explain the molecular details of this process. There are, however, many more molecular questions unanswered and further experiments are required to confirm the mechanisms presented here and to fill in the missing details.

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