REVIEW ARTICLE

Expression and Regulation of Myeloid-Specific Genes in Normal and Leukemic Myeloid Cells

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DURING THE process of myeloid differentiation, pluripotent hematopoietic stem cells become committed to myeloid precursor cells and eventually differentiate into functional, morphologically distinct end-stage myeloid cells. This process is accompanied by the coordinate expression of numerous genes. Molecular biology has made possible cloning, analysis of regulation of expression, and characterization of chromosomal structure of many of these genes. Simplistically, and for ease of experimental design, genes regulated during myeloid differentiation can be catalogued as those that code for ubiquitously expressed (“housekeeping”) proteins necessary for normal cellular proliferation and survival, for proteins important for cellular differentiation, cellular recognition and cell/cell interactions, and for gene products important for specific functions of the different end-stage myeloid cells. Cell lines established from human leukemic blasts have frequently served as homogenous and well-defined cell populations that provide both a source of nucleic acids for isolation of complementary DNAs (cDNAs) of myeloid-specific genes, and as models to study the regulation of these genes. In this review, we will focus on work that has been done to isolate and analyze genes specifically expressed in myeloid cells. This includes genes coding for secreted and cytoplasmic proteins important for microbicidal activities of these cells as well as surface molecules involved in cell adhesion and Ig binding. In addition, studies examining the patterns of expression and regulation of cytokines by normal and leukemic myeloid cells will be reviewed.

MYELOID CELL LINES: THEIR USES AND LIMITATIONS AS MODELS OF NORMAL AND MALIGNANT HEMATOPOIESIS

Myeloid leukemic cell lines established from patients with either acute myeloid leukemia (AML) or the blastic phase of chronic myeloid leukemia (CML) are arrested at different and distinct stages of myeloid development. By providing abundant amounts of cells, these lines can serve as useful models for cells representing the different steps of myeloid maturation. Many of the myeloid cell lines can be induced to partially differentiate along one or several pathways to become more mature cells. However, several features of leukemic cell lines limit their comparability with their nonleukemic cellular counterparts. These lines share with their parental, primary leukemic cells many characteristics, such as cytogenetic and molecular abnormalities that are representative of the leukemic subtype from which the lines were established. Consequently, under certain aspects they also reflect the defective, abnormal hematopoiesis as observed in leukemia. For instance, human myeloid cell lines induced to differentiate toward granulocyte-like cells always lack secondary granules. As will be shown below, regulation of several myeloid-specific genes during induced differentiation of myeloid cell lines does not fully parallel the expression patterns of these genes in normal hematopoiesis. Furthermore, some inducers of myeloid differentiation, such as retinoic acid or 1,25 dihydroxyvitamin D3 can cause simultaneous expression of both granulocyte- and monocyte-like phenotypes in these lines. Finally, investigators should remember that induced differentiation of myeloid cell lines is usually asynchronous, and often 10% to 30% of cells do not differentiate.

ESTABLISHMENT OF MYELOID LEUKEMIC CELL LINES

The most broadly studied human myeloid cell line, promyelocytic HL-60, was established from cells of a patient with AML type M2.12 These cell strains can be induced to differentiate toward cells carrying granulocytic, monocytic, eosinophilic, or basophilic markers when cultivated with certain physiologic or nonphysiologic inducers.13 14 HL-60 cells in many aspects resemble nonleukemic human promyelocytes. Because of their ease of procurement as compared with normal promyelocytes, they have frequently served as a convenient source of nucleic acids in cloning projects aimed at the isolation of cDNAs that are highly expressed at the promyelocyte stage. Similarly, macrophage- and granulocyte-like HL-60 cells have often served as sources...
for mRNAs specifically expressed in these more mature cells. In several laboratories, subclones of HL-60 cells have been developed that are resistant to the effects of a differentiation-inducing compound (eg, dimethyl sulfoxide, phorbol esters, or 1,25 dihydroxyvitamin D). These subclones are useful for dissecting the intermediate steps required for myeloid differentiation.

Other myeloid cell lines established from leukemia patients include the myeloblastic KG-1 cell line, derived from bone marrow cells of a patient with erythroid leukemia. These cells can differentiate toward macrophages when cultured in the presence of certain inducing agents. Their clonal growth is markedly enhanced by granulocyte-macrophage colony-stimulating factor (GM-CSF) and by interleukin-3 (IL-3). KG-1a is an immature myeloid subline cloned from KG-1 and resembles an early myeloblast unable to differentiate in the presence of inducers effective on more differentiated cells, including KG-1. The K562 cell line was established from a patient with CML in myeloblast crisis. These cells are arrested at a very early myeloblast/erythroblast stage. They have the potential to partially differentiate toward erythroblasts with expression of fetal globin mRNAs and synthesis of fetal hemoglobin when cultured in the presence of hemin or butyrate. After these human myeloid lines were established, many more have also been developed, thus providing a wide array of lines representing different stages of the myeloid, myelomonocytic, and monocytic lineages as well as megakaryocytic, basophil, and eosinophil cell types. Many of these cell lines are listed in Table 1. The mechanisms leading to immortalization of fresh myeloid leukemic cells after prolonged culturing are unclear, making establishment of these lines cumbersome and largely fortuitous.

EXPRESSION AND REGULATION OF MYELOID-SPECIFIC GENES

Using predominantly myeloid cell lines, many cDNAs coding for genes that are specific for functions of myeloid cells have recently been molecularly cloned and expression patterns of these genes have been determined. Table 2 lists many of the cloned myeloid-specific and myeloid-related genes, their cellular sources of cDNA, and their cellular range of mRNA expression. Availability of cDNA probes for myeloid-specific genes has made possible dissection of their developmental regulation during myeloid maturation. Again, myeloid cell lines have provided models for these different stages. This is schematized for eight genes in Fig 1.

Myeloperoxidase (MPO). MPO is an enzyme exclusively found in myeloid cells and synthesized in the azurophilic (primary) granules of promyelocytes. MPO is involved in the generation of hypochlorous acid, a potent microbicidal agent, and thus plays a key role in host defense against a variety of microorganisms. In addition, it is toxic to tumor cells, but this probably has little in vivo relevance. The mature MPO glycoprotein is a tetramer of approximately 150 Kd consisting of two 60-Kd heavy subunits and two 15-Kd light subunits. Amino acid sequence of this enzyme has been determined by virtue of the high abundance of this protein in HL-60 cells (approximately 5% of the dry weight). Others as well as ourselves have cloned human MPO cDNAs. MPO mRNA is detectable by Northern blot analysis solely in cells of the late myeloblastic and promyelocytic stages of differentiation. Normal granulocytes and monocytes express negligible amounts of MPO mRNAs although these cells have abundant MPO protein, reflecting the long half-life of this protein. Accumulation of MPO mRNA rapidly decreases upon induction of terminal myeloid differentiation of HL-60. Nuclear run-off analysis and mRNA stability studies using actinomycin D show that downregulation of MPO with terminal myeloid differentiation is controlled at least partly at the transcriptional level. However, control may also occur with processing of MPO RNA toward a pool of stable, cytoplasmic mRNA.

More recently, the chromosomal structure of the human MPO gene was elucidated. The gene consists of 12 exons spanning approximately 14 kb; it is localized on the long arm of chromosome 17 (17q22). The gene is probably not rearranged in acute promyelocytic leukemia (APL), a disease associated with a chromosomal translocation between the long arms of chromosomes 15 and 17, (t(15;17) (q22;q11.2). Regions of “open” chromatin are represented by distinct stretches of DNA with exceptionally high sensitivity to nuclease such as DNase I (“DNase I hypersensitive sites”) and are thought to represent accessibility of stretches of DNA to DNA-binding proteins regulating gene transcription. DNAse I hypersensitivity studies have examined the chromatin structure of the MPO gene. Three DNase I hypersensitive sites are present in promyelocytic HL-60 cells; they are located in the 5′ flanking region upstream of the putative MPO promoter and in close proximity of the published MPO cap site, respectively. All three of these sites are strongly diminished with induction of the cells toward granulocytic differentiation. Only one of these sites is also present in early myeloid KG-1 cells, which do not express MPO mRNA. All three sites are absent in nonmyeloid cells. This constellation suggests the presence of at least two distinct gene regions upstream of the MPO coding region that may play a role in regulation of MPO expression. One of the sites may be important in preparation of an early myeloblast to express MPO at a later stage of differentiation; the other appears to be present only in promyelocytes and may be required for active transcription of MPO. Disappearance of both sites with a decrease of MPO transcription during differentiation toward granulocytes might reflect structural changes toward “closed” chromatin.

Changes of DNA methylation in a number of genes are associated with regulation of tissue- and development-specific gene expression; demethylation of cytosine residues frequently correlates with transcription of these genes. Analysis of DNA methylation of the human MPO gene also shows expression-specific changes. Specific sites in the putative promoter region and upstream coding region of the MPO gene are demethylated in MPO-expressing normal human bone marrow cells and promyelocytic HL-60 cells, but are methylated in early myeloid cells and in nonmyeloid cells (MPO nonexpressing cells). Stepwise
demethylation of some of these sites precedes the onset of MPO expression in early myeloid cell lines. This may reflect the fact that methylation changes at an early stage of differentiation poise this gene for expression at a later maturational stage. In normal granulocytes and HL-60 differentiated to granulocyte-like cells, these sites continue to be demethylated despite a reduction of MPO transcription, implying that demethylation of the MPO gene may be necessary but is probably not sufficient for MPO expression.

MPO deficiency can be caused by an inherited genetic defect or can occur as a consequence of either myelodysplastic syndromes (MDS) or acute myelogenous leukemia. Patients with hereditary MPO deficiency may suffer from repeated infections but are often asymptomatic. In hereditary MPO deficiency, different DNA mutations probably can result in the lack of a functional MPO protein. In a report on three subjects with complete MPO deficiency and two subjects with partial MPO deficiency, expression of a normal-sized mature MPO mRNA was demonstrated in one of the individuals with complete MPO deficiency. In
Table 2. Cloning and Expression Patterns of Myeloid-Specific and Myeloid-Related Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA From</th>
<th>mRNA Expression Patterns</th>
<th>Reference</th>
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<tbody>
<tr>
<td>A. Proteins important for host defense</td>
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<tr>
<td>Myeloperoxidase</td>
<td>HL-60wt</td>
<td>P</td>
<td>63,64</td>
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<td>Eosinophil peroxidase</td>
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<td>Eo</td>
<td>76</td>
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<td>NADPH oxidase cytochrome b heavy chain</td>
<td>HL-60gran</td>
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<tr>
<td>NADPH oxidase cytochrome b light chain</td>
<td>HL-60gran</td>
<td>Mb, P, G, M, Nh</td>
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<td>NADPH oxidase cytosolic p47 protein</td>
<td>HL-60gran</td>
<td>G</td>
<td>84</td>
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<tr>
<td>NADPH oxidase cytosolic p67 protein</td>
<td>HL-60gran</td>
<td>G, M</td>
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<td>Neutrophil elastase</td>
<td>U937</td>
<td>P, Mm</td>
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<td>Cathepsin G</td>
<td>U937</td>
<td>Mm</td>
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<td>Myeloblastin</td>
<td>1F10</td>
<td>P, Mm</td>
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<td>Lysozyme</td>
<td>U937mo</td>
<td>Mm, P, G, M</td>
<td>97,98</td>
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<td>Defensins</td>
<td>HL-60wt</td>
<td>P, G, CLL cells</td>
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<td>Eosinophil major basic protein</td>
<td>HL-60gran</td>
<td>P, G</td>
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<td>Eosinophil cationic protein</td>
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<tr>
<td>B. Cytokines</td>
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<td></td>
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<tr>
<td>Granulocyte colony-stimulating factor</td>
<td>5637</td>
<td>M, G*, Fb, Nh</td>
<td>179,180</td>
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<tr>
<td>Macrophage colony-stimulating factor</td>
<td>Mia PaCa; TPA30-1</td>
<td>Mm, M, G*, Fb, Nh</td>
<td>181,182</td>
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<tr>
<td>Interleukin-6</td>
<td>TCL-NA1 (T-cell line)</td>
<td>M*, Fb, Nh</td>
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<tr>
<td>Interleukin-1α</td>
<td>Mo</td>
<td>M*, G*, Fb, Nh</td>
<td>184,186</td>
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<tr>
<td>Interleukin-1β</td>
<td>Mo</td>
<td>M*, G*, Fb, Nh</td>
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<tr>
<td>Tumor necrosis factor-α</td>
<td>HL-60mo</td>
<td>M*, G*</td>
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<tr>
<td>Interferon-α</td>
<td>KG-1</td>
<td>M*, Fb</td>
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<tr>
<td>Interferon-β</td>
<td>Fibroblasts</td>
<td>Mm, Fb</td>
<td>187</td>
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<tr>
<td>C. Receptors, adhesion molecules, and CDs</td>
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<tr>
<td>LFA-1, leukocyte adhesion protein, α-subunit (CD11a)</td>
<td>HL-60mo</td>
<td>Mm, M, T</td>
<td>188</td>
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<tr>
<td>Mo1, leukocyte adhesion protein, α-subunit (CD11b)</td>
<td>Mo, PBL, CML-PMN</td>
<td>G, M</td>
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<td>p150, leukocyte adhesion protein, α-subunit (CD11c)</td>
<td>HL-60mo</td>
<td>M</td>
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<tr>
<td>Leukocyte adhesion protein, β-subunit (CD18)</td>
<td>U937mo</td>
<td>M, G, T</td>
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<tr>
<td>FcRα (CD64)</td>
<td>PBM</td>
<td>Mm, M</td>
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<tr>
<td>FcRIIa (CDw32)</td>
<td>U937</td>
<td>Mm, Mm, P, G, M</td>
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<tr>
<td>FcRIIb-1,2 (CDw32)</td>
<td>HL-60mo</td>
<td>Mm, M, P</td>
<td>132</td>
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<tr>
<td>FcRII-1 (CD16)</td>
<td>PMN</td>
<td>G</td>
<td>133,134</td>
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<tr>
<td>FcRII-2 (CD16)</td>
<td>NK</td>
<td>NK</td>
<td>134</td>
</tr>
<tr>
<td>Leukosialin (CD43)</td>
<td>PBL + PHA</td>
<td>Mm, Mm, P, T</td>
<td>190</td>
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<tr>
<td>Aminopeptidase N (CD13)</td>
<td>HL-60wt; KG-1</td>
<td>M, G, P, G, M</td>
<td>142</td>
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<tr>
<td>CD14</td>
<td>HL-60mo; M4-AML</td>
<td>M, G</td>
<td>143-145</td>
</tr>
<tr>
<td>CD33</td>
<td>U937</td>
<td>Mm, Mm, G, M</td>
<td>139-141</td>
</tr>
<tr>
<td>CD37</td>
<td>CLL cells</td>
<td>Mm, T</td>
<td>146</td>
</tr>
<tr>
<td>M-CSF receptor (c-FMS)</td>
<td>Placeenta</td>
<td>M</td>
<td>191</td>
</tr>
<tr>
<td>IL-6 receptor</td>
<td>YT, U937</td>
<td>M, B, Nh</td>
<td>192,193</td>
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<tr>
<td>T,25 dihydroxyvitamin D_3 receptor</td>
<td>Jejunum</td>
<td>P, M, Fb, Nh</td>
<td>194</td>
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<tr>
<td>Retinoic acid receptor-α</td>
<td>MCF-7</td>
<td>P, G, Nh</td>
<td>195</td>
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<tr>
<td>Transcobalamin I (vitamin B_12 binding protein)</td>
<td>CML-PMN</td>
<td>G</td>
<td>196</td>
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</table>

(Continued on following page)

addition, four of these subjects showed abnormalities of MPO gene structure on Southern blot analysis. In view of normal-sized pro-MPO protein but lack of mature MPO protein, the underlying defect in these individuals possibly involves posttranslational processing of MPO. We analyzed the MPO gene and its expression in an individual with complete hereditary MPO deficiency.\(^{34}\) MPO expression studies on bone marrow cells from this individual using a probe for an MPO intron showed accumulation of heterogeneous nuclear MPO RNA, whereas no mature MPO mRNA was detectable in these cells using an MPO cDNA probe and no mature MPO protein was seen using Western blot analysis. These observations, together with Southern blot data indicating no gross abnormality of this gene, suggest an RNA splicing defect, eg due to a point mutation or a small deletion possibly located near an intron-exon border. This type of alteration is also the most frequent molecular defect seen in thalassemias.\(^{34}\) We are now directly sequencing the MPO gene in these patients using the polymerase chain reaction (PCR) to identify the specific defect(s). We suspect that a variety of mutations in the MPO gene result in MPO deficiency.

Neutrophil NADPH oxidase. The multicomponent enzyme neutrophil NADPH oxidase is activated in stimulated granulocytes and catalyzes the production of superoxide. It has an important function in host defense, as evidenced by
Studies by several laboratories showed that mRNA interferon-α (IFN-α) or tumor necrosis factor-α (TNF-α) have been studied. The transcript is absent in nonhematopoietic tissues. Some patients with X-linked CGD do not have detectable transcripts coding for the heavy subunit of membrane-bound neutrophil cytochrome b, their expression and regulation in myeloid cells have also been studied.

The 91-Kd heavy subunit is encoded by a gene also termed X-CGD (chronic granulomatous disease) gene. Its transcript is expressed in a tissue-specific manner; levels of expression are very low in promyelocytic HL-60 cells but are strongly upregulated with induction of these cells toward granulocyte-like or monocytic differentiation. The gene is also expressed in normal monocytes and granulocytes. In contrast, B lymphocytes express low level of X-CGD mRNA, and the transcript is absent in nonhematopoietic tissues. Some patients with X-chromosome linked CGD do not have detectable transcripts coding for the 91-Kd subunit in their leukocytes, and gross alterations have been found in the respective gene. In a variant form of CGD, the patients' cells express a normal-sized transcript, but sequencing of the coding region of the gene shows an isolated point mutation resulting in a nonfunctional p91.

Myeloid cell lines have served as models in studies addressing the regulation of the gene coding for the p91. Studies by several laboratories showed that mRNA accumulation of this gene is strongly upregulated in HL-60, U-937, THP-1, and ML-3 after treatment of the cells with interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α). Added together, these cytokines act synergistically. Nuclear run-off experiments show that the effects of each of these compounds as well as of a combined regimen of retinoic acid and dimethyl formamide predominantly stimulate transcription of the X-CGD gene.

A cDNA for the 22-Kd light subunit of membrane-bound cytochrome b has also been cloned. This gene codes for a 0.8-kb mRNA expressed in promyelocytic and granulocytic HL-60 cells as well as in K562 myeloid cells, in B cells, and several types of nonhematopoietic cells. The transcript, in contrast to that of the heavy subunit of cytochrome b, therefore is not restricted to cells with NADPH oxidase activity, its expression is constitutive and is not significantly altered by regimens that modulate expression of the heavy subunit.

Very recently, cDNAs for both the 47- and 67-Kd proteins that are part of the cytoplasmic component of NADPH oxidase were isolated from libraries constructed from HL-60 cells induced toward granulocyte-like differentiation. These mRNAs are also expressed in normal granulocytes and monocytes. However, both mRNA and protein of the 67-Kd subunit are absent in cells from many patients with the autosomal form of CGD.

**Myeloid-specific serine esterases (neutrophil elastase, cathpsis G).** Human neutrophil elastase (NE) is a serine protease that digests elastin and collagens as well as a variety of other human proteins. Cellular localization and release of NE protein parallel that of MPO; NE is stored in azurophilic (primary) granules of PMNs and released into the extracellular environment after activation of these cells. A cDNA for NE was recently cloned from a library made from U937 cells. Analysis of expression of NE mRNA in normal human myeloid cells and myeloid cell lines shows...
several general similarities but also subtle differences when compared with the regulation of expression of the MPO gene. mRNAs for both NE and MPO accumulate to high levels in promyelocytes, but both are absent in mature PMNs, macrophages, and in macrophage-like HL-60 cells induced with a phorbol ester for 3 days. However, mRNA expression of NE is upregulated in HL-60 cells treated with dimethyl sulfoxide, whereas rapid shut-off of MPO occurs in HL-60 treated similarly. This implies that the mechanisms regulating expression of both genes are complex and suggests that peak levels of NE transcripts in HL-60 cells are reached after these cells have partly differentiated toward granulocytes. The gene for NE has been isolated, characterized, and its chromosomal location has been determined. It is composed of 5 exons covering 4 kb and is located on the long arm of chromosome 11q14. The 5' flanking region of the NE gene harbors a 19-bp pyrimidine-rich sequence sharing 95% homology with a sequence present in the 5' flanking region of the MPO gene. Possibly, this sequence is functionally related to the observed similarities in expression patterns of NE and MPO.

Human cathepsin G is a serine protease of 26-Kd molecular weight that accumulates in the azurophilic granules of neutrophils and monocytes. Like NE, cathepsin G is probably involved in the breakdown of connective tissue proteins. A cDNA coding for cathepsin G was isolated by Salvesen et al using myelomonocytic U937 cells. Macrophage-like differentiation of the cells using a phorbol ester decreases mRNA expression of cathepsin G as a result of decreased transcription of the gene. Cloning of the cathepsin G gene showed a structure that is similar to the NE
gene and other serine proteases; in addition, several motifs in the 5' flanking region are identical to sequences in the genes for NE and MPO. In situ hybridization, the cathepsin G gene was mapped to chromosome 14, band q11.2.

**Lysozyme.** Lysozyme, like MPO, is an enzyme primarily involved in host defense. It hydrolyzes the linkage between bacterial N-acetylmuramyl acid and N-acetyl-D-glucosamine. Human lysozyme protein has been characterized and its tissue distribution has been analyzed. It is most abundant in macrophages, accounting for 2.5% of total protein in these cells. DNAs complementary to human lysozyme have recently been cloned. Several studies have shown that accumulation of lysozyme mRNA is high in normal monocytes. On in vitro maturation to macrophages, these cells increase lysozyme expression. Compared with monocytes and macrophages, this mRNA is less abundant in U937 myelomonocytes and HL-60 promyelocytes and induction of monocytic differentiation of these two cell lines using phorbol ester results in decreased levels of lysozyme mRNA (reference 98 and Lubbert M, Chung LP, Mertelsmann R, Herrmann: unpublished observation, October, 1990), which suggests a partially defective differentiation program in these cells. In contrast, Redecker et al. have examined the regulation of lysozyme transcription using myelomonocytic U-937 cells and the macrophage-inducer 1,25 dihydroxyvitamin D3. This compound induced a more than 10-fold increase in expression of lysozyme transcripts, and nuclear run-off experiments showed that this increase was regulated predominantly at the transcriptional level. These studies show that at times, the same cell line can respond dramatically different to two different inducers of macrophase-like differentiation.

Very recently, the genomic structure and chromosomal localization of the human lysozyme gene have been elucidated. The gene consists of 4 exons spanning an approximately 6-kb region on chromosome 12.

**Defensins.** Defensins (also termed human neutrophil peptides, HNP) are a family of peptides of 29 to 34 amino acids that are part of the nonoxidative host defense apparatus of mature granulocytes. Several human defensin peptides have been isolated and characterized by amino acid sequencing. HNP 1-3 are stored in the primary granules of mature neutrophils and make up about 30% of the total granule protein of these cells. cDNAs for two of these human defensins have been cloned. Two myeloid-specific cDNAs known as mrs and pcG14 have been cloned and have been shown to code for defensins HNP-1 and HNP-3. Their nucleotide sequence provides evidence that each defensin originates from a separate 94-amino acid precursor protein, which is subsequently processed to yield a mature peptide. Expression studies showed that defensin mRNA is detectable in unfractionated normal bone marrow cells, in leukemic cells from patients with AML, CML and, surprisingly, with chronic lymphocytic leukemia (CLL), but not in normal mature neutrophils, lymphocytes, or monocyes. These results imply that defensin mRNA accumulation is highest in neutrophil precursor cells but not terminally differentiated normal neutrophils. Defensin mRNA present in HL-60 promyelocytes increases up to sixfold with induction of these cells toward granulocytes. This might mean that this gene is upregulated during progression of differentiating promyelocytes toward the myelocyte and metamyelocyte stages.

**Lactoferrin.** Lactoferrin is an approximately 80-kDa iron-binding protein synthesized by myeloid cells as they progress through the myelocytic to late metamyelocytic stages of development; the protein is stored in the secondary ("specific") granules of these cells. Its function is as yet unclear, but may be associated with regulation of proliferation of myeloid cells. However, expression of this protein is not restricted to myeloid cells: lactoferrin is also synthesized by several secretory tissues such as the pancreas, salivary gland, and mammary tissue. Lactoferrin cDNAs were recently isolated. Northern blot analysis shows that lactoferrin-specific transcripts can be detected in bone marrow cells from healthy donors and from patients in the stable phase of CML. However, less mature myeloid cells lack lactoferrin transcripts. Interestingly, two promyelocytic human cell lines (HL-60, PLB-985) that can be induced to partially differentiate toward granulocytes are unable to express lactoferrin RNA even after induction toward granulocytic or monocytic differentiation. These results confirm the lack of lactoferrin protein in granulocytic HL-60 cells and are consistent with the notion that myeloid differentiation as seen in the HL-60 cell model is incomplete or defective in some aspects. To date, no human myeloid cell line has been shown to express proteins found predominantly in secondary granules (lactoferrin, transcobalamin I). This fact might imply that these lines have altered expression of a transcription factor important in the global regulation of genes coding for proteins present in the secondary granules. In contrast, several murine myeloid cell lines are capable of expression of proteins of the secondary granules and are probably appropriate models to use for studying regulation of these genes.

Recently, abnormal regulation of lactoferrin gene expression has been demonstrated in bone marrow cells from two patients with neutrophil specific granule deficiency (SGD). These patients have dramatic reduction of expression of lactoferrin mRNA in their bone marrow cells. Also, these cells contain no lactoferrin protein despite normal secretion of lactoferrin into nasal fluid of one of these patients. Lactoferrin proteins made by both neutrophils and glandular epithelia are probably encoded by the same gene. Thus, the defect leading to lack of neutrophil lactoferrin and other secondary granule proteins in patients with SGD might also be due to disturbed coordination of expression of this group of genes in myeloid precursor cells during myeloid maturation, again possibly due, for instance, to lack of a key trans regulatory protein.

**CD11b/CD18 (Mo1, Mac-1).** The adhesion molecule CD11b/CD18 (also termed Mo1 or Mac-1) is a member of a family of three heterodimeric cell surface glycoproteins and thus part of the broad gene family of integrins involved in cell-cell interactions during host defense and immune response. CD11/CD18 surface molecules share a common /3-subunit (CD18, Mac-1 /3, Mo1 /3) of 95-Kd molecular...
weight that is noncovalently linked to a unique ζ-subunit of higher molecular weight. The ζ-subunits have been designated CD11a (LFA-1, α L), CD11b (Moα, Mac-1, α M), and CD11c (p150, α X), respectively. The structure and function of these molecules have recently been reviewed. Therefore, we will limit our discussion to CD11b/CD18, which is an important marker of terminal myeloid differentiation because of its high specificity for myeloid cells.

The CD11b/CD18 surface antigen is first expressed during the myelocytic and monoblastic stages of maturation and is upregulated during granulocytic and monocytic differentiation. Functions of this molecule include binding of a complement component (C3b fragment) as well as promotion of homotypic granulocyte adhesion and adhesion of granulocytes and monocytes to endothelial cells. In addition, CD11b/CD18 molecule is associated with granulocytic oxidative burst, phagocytosis, and antibody-dependent cellular cytotoxicity (ADCC). These important functions are exemplified by the fact that patients with CD11b/CD18 deficiency suffer from recurrent infections (see below).

Recently, cDNAs for both the α-subunit and β-subunit of CD11b/CD18 have been isolated by several groups and their chromosomal localizations have been determined. The gene for CD11b resides on chromosome 16p11.13 clustered with CD11a and CD11c, and the CD18 gene is located on chromosome 21q22.120 Expression of CD11b mRNA is sharply upregulated during induction of granulocytic or monocytic differentiation of HL-60.117 This upregulation of CD11b transcripts is paralleled by an increase in both the number of CD11b molecules per cell as well as the number of CD11b-positive cells. In a similar manner, expression of transcripts for the β-subunit CD18 is upregulated during chemical induction of HL-60 to granulocytes or monocytes.121 Run-off transcriptional analysis of nuclei from both promyelocytic and retinoic acid-treated granulocytic HL-60 showed that the mechanism of upregulation of CD18 is transcriptional.121

Molecular defects in the gene coding for CD18 have been associated with the rare occurrence of hereditary deficiency of leukocyte adhesion molecules (Leu-CAM deficiency). Heterogenous genetic alterations including deletions or point mutations result in a clinical picture of recurrent and often fatal bacterial infections, emphasizing the important role of CD11/CD18 molecules in normal granulocyte functions.

**Receptors for IgG.** Receptors binding the Fc fragment of IgG in complex with antigen target these complexes to the effector cells. Thus, these receptors (FcR) represent a link between the humoral and cellular immune responses. Three types of Fc receptors for the constant region of IgG have been recogzed based on their different affinities for IgG and their tissue-specific expression patterns. FcRI (CD64) is a high-affinity receptor expressed by monocytes, FcRII (CDw32) is a low-affinity receptor found on various hematopoietic cells including granulocytes, monocytes, B cells, and platelets; FcRIII (CD16) is a low-affinity receptor, its expression is restricted to macrophages, neutrophils, natural killer (NK) cells, and T-suppressor cells. Recently, cDNAs for these three groups of human Fc receptors have been cloned. These cDNAs have served as tools for determining the expression patterns of this group of receptors and for dissecting the molecular mechanisms responsible for their diversity of function. For instance, expression studies using a cDNA for the high-affinity receptor FcRII showed expression in normal monocytes, in myelomonocytic U937 cells, and in placental tissue (rich in monocytes/macrophages). Similarly, expression of mRNA for the low-affinity receptor FcRIII (CD16) was demonstrated in cells that also express the receptor protein, ie, peripheral blood mononuclear cells and NK cells but not in myelomonocytic U937 cells or in several lymphoblastoid cell lines. However, significant biochemical and structural differences with regard to molecular weight and anchorage in the cellular membrane exist between the FcRIII expressed on granulocytes versus NK cells. The molecular basis for these differences was elucidated by Ravetch and Perussia by sequence analysis of FcRIII mRNA from these two cell types using PCR. This type of analysis showed several cell type-specific nucleotide changes resulting in two transcripts that are expressed in a mutually exclusive manner. One of these nucleotide substitutions results in exchange of a translation termination codon (present in PMN-specific transcripts) by a codon coding for arginine in NK-specific transcripts. This alteration leads to an extension of the open reading frame by an additional 21 amino acids and thus accounts for the higher molecular weight of the NK-specific FcRIII protein and a cytoplasmic domain that is longer by 21 amino acids in NK cells than its homologue in PMNs. This finding was confirmed by cloning two genes for FcRIII that share a high degree of homology, except for the amino acid changes characteristic in their respective transcripts. Both of these genes are localized on chromosome 1.

The low-affinity receptor FcRII is expressed most broadly, being found on various cells of both the myeloid and lymphoid lineages. Similarly, binding of immune complexes to their receptor elicits diverse cellular responses such as ADCC, phagocytosis, and the release of cytokines. Cloning of several types of human FcRII cDNAs has elucidated part of this functional heterogeneity. Brooks et al isolated and characterized these cDNA clones from U937, from monocyte-like HL-60, and from B-lymphoid Daudi cells. This analysis resulted in the identification of several distinct clones expressed in an overlapping but distinct manner and coding for five different receptor molecules. Genomic cloning showed that these five cDNAs are encoded by at least three different genes. Therefore, these studies allowed for formulation of a model that integrates both conservation and divergence in the evolution of FcRII receptors with overlapping but distinct structures, thus accounting for the diverse biologic effects of ligand binding to this class of receptors.

**Other differentiation antigens.** For some of the cell surface antigens found on hematopoietic cells and grouped as “cluster determinants” (CDs), no specific functions can be assigned as yet. Still, these differentiation antigens yield valuable tools for characterization of different hematopoietic cell populations. Many of these proteins have also
been molecularly cloned, and analyses of gene expression and structure have been performed. The surface antigens expressed on myeloid cells and analyzed thus include the CD34 marker molecule for human hematopoietic stem cells, a marker for colony-forming units for granulocytes, erythrocytes, monocytes, and megakaryocytes (CD33), for progenitor cells of the granulocytic and monocytic lineages (CD13), and for mature monocytes (CD14). mRNA expression of these molecules has been analyzed in established myeloid cell lines (see Table 2 and Fig 1). Analysis of the cDNA sequence of CD13 showed its identity to the aminopeptidase N that is present on membranes of cells of the small intestine, kidney, and placenta. In addition, the structure of genes coding for these molecules and their chromosomal localization have been elucidated: CDS 34, 33, 13, and 14 are localized on chromosome 1 (1q12-1qter), 19 (19q13.3), 15 (15q25-26), and 5 (5q23-31), respectively. Run-off analysis and mRNA stability experiments have been used to dissect the regulation of CD34 mRNA expression with induced monocytoid differentiation of KG-1 cells and showed a posttranscriptional mode of downregulation.

REGULATION OF CYTOKINE EXPRESSION BY MYELOID CELLS

Many of the proteins collectively termed cytokines physiologically are either acting on myeloid cells, expressed by them, or both. Expression of some of these cytokines is restricted to one cellular lineage, whereas others are expressed by many different cell types. cDNAs from several cytokines have been molecularly cloned from myeloid cells (Table 2).

TNF-α. TNF-α is a 17-Kd protein synthesized by activated monocytes and granulocytes. It has a broad spectrum of functions, including cytotoxicity to tumor cells and immune modulation through induction of cytokines in fibroblasts, endothelial cells, and monocytes. A TNF-α cDNA was first cloned from HL-60 cells induced with a phorbol diester. Analysis of TNF-α expression in myeloid cells has shown expression of low levels of TNF-α mRNA in promyelocytic HL-60 cells and in U937 cells. mRNA levels are strongly increased with monocytic induction of these cells. This upregulation is predominantly mediated by an increase in transcription, as shown by transcriptional run-on analysis using HL-60 cells induced to differentiate by 1,25 dihydroxyvitamin D₃ or normal monocytes stimulated by a phorbol diester.

M-CSF-receptor (c-FMS). The receptor for M-CSF (CSF-1) is encoded by the c-FMS proto-oncogene. Expression of this receptor molecule is developmentally regulated, with highest levels in normal human peripheral blood monocytes and in HL-60 cells induced toward monocytic differentiation. In contrast, c-FMS is undetectable or only poorly expressed in mature granulocytes or in myeloid cells less mature than HL-60. Expression of c-FMS is also seen in primary leukemic cells from patients with different types of AML. Uptregulation of this gene in TPA-induced HL-60 cells is regulated predominantly posttranscriptionally. In nuclear run-off experiments, it is constitutively transcribed in HL-60 cells in the absence of accumulation of its mRNA. Stabilization of this mRNA in HL-60 as the cells differentiate toward monocytes is mediated by a labile protein, as demonstrated by a reversal of stabilization after pretreatment of these cells with an inhibitor of new protein synthesis.

Transcription of cytokines in activated granulocytes. The mature granulocyte had previously been thought to be a transcriptionally inactive end-stage cell. However, when stimulated with GM-CSF or phorbol ester these cells can be induced to actively transcribe the c-fos proto-oncogene as well as a number of cytokines, including IL-1, IL-6, IFN-α, TNF-α, G-CSF, and M-CSF, and to secrete biologically active G-CSF, M-CSF, and IL-6 protein. These observations suggest that functionally, the granulocyte is related to the peripheral blood monocyte, which is also able to express and secrete a variety of cytokines ("monokines") constitutively and on activation.

Autocrine growth stimulation in myeloid leukemic cells. Primary blast cells from some patients with AML express and secrete functionally active cytokines, including G-, GM-, M-CSF, IL-1 β, IL-6, and TNF-α. Some investigators have interpreted these findings to suggest that acute myelogenous leukemic cells in a subpopulation of patients may have developed autocrine stimulation to spur their own growth, thereby escaping physiologic homeostasis of proliferative control. The mechanism(s) by which this aberrant expression of cytokines in their transformed myeloid cells is mediated may involve stabilization of their mRNAs. An analysis of transcription and mRNA stability has found that cells from many AML patients transcribe CSFs, but much fewer accumulate CSF mRNA. These latter samples are unique in having a marked stability of their CSF mRNAs. Infrequently, alterations of human cytokine genes as detected by Southern blot analysis have been reported. Stocking et al isolated growth-factor–independent mutant cell clones from the growth-factor–dependent murine promyelocytic D35 cells. Molecular analysis of these mutants showed abnormal expression of the GM-CSF or IL-3 genes and concomitant secretion of these growth factors in an autocrine fashion. Activation of the GM-CSF gene in three cell clones was demonstrated to be a result of insertion of transposable-like viral elements at different regions of the GM-CSF gene. Similar activation of cytokine genes has been reported for FDC-P1 cells that had been injected into irradiated mice. Activation of cytokine genes (or other genes) through viral insertion has never been reported in human myeloid leukemia cells.

Introduction of the gene for IL-3 into murine hematopoietic progenitor cells by retroviral gene transfer has been performed by several laboratories. Recipient mice have elevated serum levels of IL-3 activity and exhibit a syndrome bearing resemblance to the chronic phase of CML: splenomegaly, dramatic peripheral blood leukocytosis, hyperplasia of the granulocytic lineage in the bone marrow, but normal indices of myeloid differentiation. None of these animals developed acute leukemia. Similarly, introduction of the murine G-CSF gene into murine bone marrow cells using retroviral gene transfer and implantation of
these cells into recipient mice resulted in non-neoplastic expansion of neutrophils in these cells.\(^7\)

**CONCLUDING REMARKS**

Application of recombinant DNA technology has made possible cloning and expression analyses of multiple genes important for and germane to myeloid cells. These studies were greatly facilitated by the availability of a wide array of myeloid leukemic cell lines arrested at different stages of myeloid differentiation. Regulation of many myeloid-specific genes is controlled at the level of transcription, and several groups of genes (eg, those for proteins for either primary or secondary granules) are coregulated. Further studies using information about the regulatory regions upstream of these genes, eg, those derived from chromatin structure analysis and sequence information, will be necessary to define binding sites for the different transcriptional regulator proteins that act in concert during myeloid differentiation and will eventually result in purification and characterization of these factors as well as their interplay. Similarly, myeloid cell lines will provide a source for purification and cloning of myeloid-specific transcription factors.

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