Interleukin 3-dependent Proliferation of the Human MO-7e Cell Line Is Supported by Discrete Activation of Late G1 Genes

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Abstract

The hemopoietic growth factor interleukin 3 (IL-3) supports the survival and proliferation of multipotent and committed progenitor cells in vitro. To elucidate the molecular mechanisms triggered by IL-3 we studied the expression of cell cycle-related genes in a recently established human IL-3-dependent clone (M-07e). No changes in the level of expression of early (c-myc), mid (ornithine decarboxylase), or mid-late G1 (p53, c-myb) cell cycle genes were detected after restoration of IL-3 in deprived cells. The fact that only late G1-S-phase genes [proliferating cell nuclear antigen (PCNA) thymidine kinase (TK), histone H3] are modulated by IL-3 suggests that this factor may control human cell proliferation by acting at the G1-S boundary.

Introduction

Both normal hemopoietic progenitors and GF3-dependent lines require a continuous supply of GF for in vitro survival and proliferation (1, 2). Deprivation blocks their proliferation and decreases their metabolism (3), whereas it merely drives fibroblasts into quiescence (G0) without altering their viability. Restoration of GF reverses these effects, whereas continued starvation triggers apoptosis (4).

We have recently established a subclone named M-07e (5), strictly dependent on the presence of IL-3 or granulocyte-macrophage colony-stimulating factor for survival and proliferation from a human megakaryoblastic cell line (6) bearing antigen determinants (CD13, CD33, and CD34) common to multilineage hemopoietic precursors. Withdrawal leads to a sharp fall in its proliferative activity to a nadir within 24 h after GF deprivation. However, after 48 h of deprivation, cell viability is reduced by only about 40%, and GF restoration fully restores proliferation. To elucidate the mechanisms by which IL-3 supports the proliferation of human hemopoietic cells we investigated the GF-dependent regulation of several cell cycle-related genes in M-07e cells.

Materials and Methods

Cells. M-07e cells were obtained from the original M-07 human megakaryoblastic cell line (6) as described (5) and routinely grown as a suspension culture in IMDM supplemented with 5% fetal calf serum and 5 ng/ml of recombinant human IL-3 (a gift from Genetics Institute, Cambridge, MA). Growth factor deprivation was achieved by washing the cells twice in IMDM and culturing them for 48 h in IMDM supplemented with 5% fetal calf serum. Proliferation was restored by adding 5 ng/ml of IL-3.

Proliferation Assay. To monitor changes in the proliferative activity after restoration of IL-3, 1-ml aliquots of M-07e cell suspensions were pulse labeled with 1 µCi/ml of [3H]thymidine for 1 h at different time intervals. The fraction of cells in S phase was assessed by autoradiography on cytoospin preparations processed by standard methods.

Northern Blot Analysis. Total RNA was extracted with guanidinium thiocyanate and isolated by acid phenol-chloroform extraction (7). Polyadenylated mRNA was obtained by affinity chromatography on oligodeoxythymidylic acid cellulose columns according to the method of Sambrook et al. (8).

RNA (20 µg) or polyadenylated RNA (4 µg) samples were fractionated on 6% formaldehyde-1.2% agarose and blotted onto nitrocellulose membranes. Filters were hybridized to 32P-labeled random-primed DNA probes according to the method of Sambrook et al. (8), washed for 30 min in 0.1 x standard saline-citrate/1% sodium dodecyl sulfate at 52°C, and exposed to X-ray film for 2-4 days. The following probes were used: c-myc was a nearly full-length human cDNA (9); p53 was a murine cDNA fragment (10); c-myb was a nearly full-length human cDNA (11); PCNA was a human cDNA (12); TK was a nearly full-length human cDNA (13); histone H3 was a human cDNA (14); and ODC was a nearly full-length human cDNA (15).

ODC Enzymatic Activity. ODC enzymatic activity was determined as the amount of 14CO2 released from 0.5 µCi DL-[1-14C]ornithine, as described by Farrar (16). Released 14CO2 was trapped by KOH as KHCO3 and evaluated by scintillation counting. 14CO2 evolution is expressed as pmol 14CO2/h/106 cells.

Results

The expression of c-myc, c-myb, p53, ODC, PCNA, TK, and H3 genes was studied at different times after addition of IL-3 to deprived cells. The results demonstrate that IL-3 stimulation does not change appreciably the level of expression of c-myc, c-myb, and p53 in the time lapse between IL-3 addition and the peak of DNA synthesis (Figs. 1a and 2c). Similarly, the lack of increase of ODC mRNA during the first 12 h after stimulation by IL-3 demonstrates that it does not induce early active transcription of this gene (Fig. 2a). However, the presence of a significant reduction of ODC mRNA 3 and 6 h after stimulus (Fig. 2a, Lanes 3 and 4) together with the concurrent increase of ODC activity (Fig. 2b) suggest that IL-3-induced ODC activity is caused by translational activation of ODC mRNA. This interpretation is confirmed by the finding that addition of actinomycin D does not affect the early rise of ODC activity but fully inhibits its later increase, while the addition of cycloheximide always exerts an inhibitory effect (Fig. 2b).

As shown in Fig. 1b, an increase in PCNA mRNA expression was detected 4 h after GF restoration and reached a maximum between 8 and 16 h. This increase preceded the synthesis of TK and H3 mRNAs. Moreover, as shown by the [3H]thymidine

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level of c-myc transcript is not affected by the addition or deprivation of IL-3, indicating that the myc pathway of nuclear activation is not involved in the events triggered by IL-3 binding to M-07e cells. Moreover, according to previous observations (17-19), constant c-myc activation does not abrogate the requirement of M-07e cells for IL-3, suggesting that c-myc transcription alone is not sufficient to sustain independent growth.

In some murine IL-3-dependent cell lines, the myc-ODC activation sequence has been proposed as the molecular mechanism of IL-3-sustained cell proliferation (2, 16). This is not the case in M-07e cells; we did not observe any increase of

pulse labeling studies, the expression of these two genes is closely linked to DNA synthesis (Fig. 2c).

Discussion

The IL-3 gene activation pattern in M-07e cells differs substantially from that of murine IL-3-dependent cell lines. The

Fig. 1. Northern blot analysis of cell cycle-related gene expression. a, analysis of c-myc, p53, and c-myb RNA from M-07e cells at different times after IL-3 restoration showing an unchanged level of gene expression from 0 to 24 h; b, analysis of PCNA, TK, and histone H3 RNA from M-07e cells treated as in a showing transcriptional activation peaking at 8, 16, and 24 h, respectively. Molecular size of specific transcripts in kilobases (kb) are indicated, as well as the 18S and 28S marker RNA. Top abscissa, time (h) after IL-3 addition at which the cultures were sacrificed and RNA was extracted.

Fig. 2. IL-3-induced ODC gene expression and enzymatic activity in relation to the entry into S phase of M-07e cells. a, Northern blot analysis of ODC gene expression from M-07e cells at different times after IL-3 restoration shows a decreased level of expression at 3 and 6 h and a subsequent rise starting from 12 h; time course of ODC enzymatic activity of M-07e cells treated as in a in the presence and absence of cycloheximide (CH) (20 μg/ml) and of actinomycin D (AMD) (50 ng/ml). The early rise of ODC activity is not affected by actinomycin D, while cycloheximide completely inhibits it at any time; c, percentage of M-07e cells in DNA synthesis at different times following IL-3 restoration. Molecular size of specific transcripts in kilobases (kb) is indicated, as well as the 18S and 28S marker RNA. Top abscissa, time (h) after IL-3 addition at which the cultures were sacrificed. RNA was extracted, ODC activity was assayed, and the labeling index was evaluated.
ODC transcript until 12 h after addition of IL-3, raising the possibility that ODC gene activation is due to a feedback mechanism, rather than to direct induction of transcription by IL-3. This view is confirmed by the data from ODC activity in cells in which protein synthesis or RNA transcription were impaired with the addition of cycloheximide or actinomycin D, respectively; cycloheximide, but not actinomycin D, blocks the early increase of ODC activity after IL-3 addition, suggesting a possible posttranscriptional regulation of the ODC gene by IL-3.

Several models have recently supported the hypothesis that late G1 events play a major role in the control of cell proliferation. When some G1-arrested, temperature-sensitive mutants of the cell cycle (20,21) are induced to overcome the block, they activate a subset of late G1 or G1-S boundary genes. The cdc2 gene, first described in the fission yeast (22) and recently cloned in humans (23), encodes a function essential at the end of the G1 and in G2. The M-07e cell gene expression pattern suggests that deprivation blocks the cells in a late G1 phase and that in this system, too, critical events in the late G1 period control cell proliferation.

It has been shown that IL-3 does not activate normal quiescent hematopoietic cells to proliferate, but it is needed as a progression factor supporting their proliferation after their stimulation by IL-1 and IL-6, which seem to act as competent factors (24). Our finding that IL-3 does not affect the activity of G0-G1 transition genes such as c-myc and of mid-G1 genes demonstrates that IL-3 also represents a progression factor for M07e cells. The pattern of gene expression suggests that the mechanism by which IL-3 sustains proliferation may be the simple maintenance of an active transcriptional state of the genes of the G1-S boundary. Whether this pattern is unique to the M-07e cell line or is shared by normal hematopoietic cells has to be established.

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References
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