Comparison of Factors Which Induce Differentiation of the Murine Myeloid Leukaemic Cell Line M1 *

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A. Introduction

Naturally occurring regulatory molecules which can cause the terminal differentiation of leukaemic cells have recently been the subject of great interest, and to date, several protein factors have been described that can induce the differentiation of various myeloid leukaemic cells in vitro. The haematopoietic colony-stimulating factors, granulocyte-CSF (G-CSF) and granulocyte/macrophage-CSF (GM-CSF) [1, 2], which act as both proliferative and differentiative stimuli for normal myeloid progenitors, are able to induce the differentiation of certain murine and human myeloid leukaemic cells (including murine WEHI-3BD$^+$ and M1 and human HL-60) [3–5]. By contrast, a number of other activities have been described which are capable of inducing the differentiation of murine M1 leukaemic cells, yet which do not stimulate the proliferation of normal progenitor cells [6–8]. With the recent cloning of myeloid leukaemia inhibitory factor (LIF [22]; = D-factor [6]), they have all been cloned. In this report the genetic and polypeptide structures of LIF are compared with those of two growth factors also known to cause differentiation of M1 cells: G-CSF and interleukin-6 (IL-6; MGI-2).

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B. Genetic and Polypeptide Structures of M1 Differentiation-inducing Factors

I. G-CSF

Natural and recombinant G-CSF can induce the terminal differentiation of M1 leukaemic cells [9]. Genomic and cDNA sequences for both human and murine G-CSF have been reported [10, 11]. The genes are highly homologous and the encoded proteins display 76% identity with three insertions or deletions [11]. Murine G-CSF consists of 178 amino acids with a calculated Mr of 19061 and is O-glycosylated to an Mr of approximately 24000–25000. Comparison of the structures of human G-CSF cDNAs and the chromosomal gene revealed that there is a possibility of two different G-CSF molecules consisting of 177 or 174 amino acids [10], corresponding to a calculated molecular mass of 18671 for the smaller version. Native human G-CSF is O-glycosylated to approximately 21000 daltons [12]. Neither human nor murine G-CSF have any N-linked glycosylation sites, and each molecule is presumed to be intramolecularly linked via two disulphide bonds [11].

II. IL-6 [MGI-2]

Over the past few years, IL-6 has been variously known as B-cell stimulatory factor [13], interferon-β2 [14], 26-kD inducible protein [15], hybridoma/plasmacytoma growth factor [16] and interleukin-HP1 [17]. IL-6 also stimulates the differentiation of B cells [13] and has some activities as a GM-colony-stimulating factor [18]. To this variety of seemingly unrelated activities (and more; see [19])

can be added its ability to cause the terminal differentiation of M1 leukaemic cells (D. Metcalf, personal communication).

Human and murine IL-6 have been cloned [13–15, 20] and show low sequence conservation (42% between proteins, with five insertions or deletions [20]), especially in the amino terminal half. Human IL-6 consists of a 184-amino acid peptide of calculated mass 20781 which is presumed to be glycosylated to its observed native Mr of 26000 via two potential N-linked and four potential O-linked glycosylation sites [13]. Murine IL-6 consists of 187 residues with a calculated Mr of 21710 which is variably O-glycosylated to between 22000 and 29000 daltons [20, 21].

The primary sequence of the IL-6 protein revealed significant homologies with the sequence of G-CSF [13], including the conservation of four cysteine residues. Comparison of their gene sequences suggested that the two genes may be derived from a common ancestoral gene [13]. Recently, it has been demonstrated that the sequence of at least one form of purified MGI-2 [7] is the same as that of murine IL-6 (A. Zilberstein and L. Sachs, personal communication).

III. LIF [D-Factor]

LIF, a factor that we have recently purified [8] and cloned [25, 26] is able to induce the differentiation of M1 cells but, unlike G-CSF, not WEHI-3BD$^+$ cells [8]. LIF is of particular interest for leukaemia therapy, because, unlike the CSFs and IL-6, it does not appear to be a proliferative stimulus for either normal or leukaemic progenitor cells [8]. In addition, LIF is unique in that at high concentrations it inhibits M1 colony formation [8]. LIF is one of the most highly conserved of the regulators known to act within the myeloid system. The mature murine and human protein sequences share 78% identity (with no insertions or deletions) [23], and consist of 179 amino acids. Murine LIF is heavily glycosylated from its calculated protein mass of ~20000 daltons to approximately 58000 daltons, via mostly potential N-linked and some potential O-linked glycosylation sites [22]. The murine and human LIF molecules share six conserved cysteine residues. D-factor [6] has recently been purified and its determined amino acid sequence is the same as that of murine LIF (M. Hozumi, personal communication).

C. Is LIF Related to G-CSF and IL-6?

Given that G-CSF and IL-6 appear to be related in both polypeptide and gene arrangements [13, 24], it is interesting to consider whether LIF has any relationship to these two regulators.

I. Protein Sequence/Structure

LIF shares little detectable homology with G-CSF and IL-6 when their protein sequences are compared. Furthermore, the number and positions of the cysteine residues in the LIF proteins are not equivalent to those of G-CSF and IL-6 (Fig. 1), and whilst G-CSF and IL-6 are minimally glycosylated (mostly O-linked) [11–13, 20], LIF is heavily N-glycosylated [8, 22, 23]. However, murine and human LIF share two small patches of homology with murine and human G-CSF and minor similarities may be observed in the distribution of proline clusters in all three molecules, and each is of a similar length. Thus, at the polypeptide level LIF bears only little resemblance to G-CSF and even less to IL-6.

II. Gene Organization

Comparison of the gene organization of LIF with that shared by G-CSF and IL-6 reveals little similarity (Fig. 2). The LIF genes comprise three exons compared with the five exons of both G-CSF and IL-6 and, apart from the coding region of the first exon of murine LIF, which is the

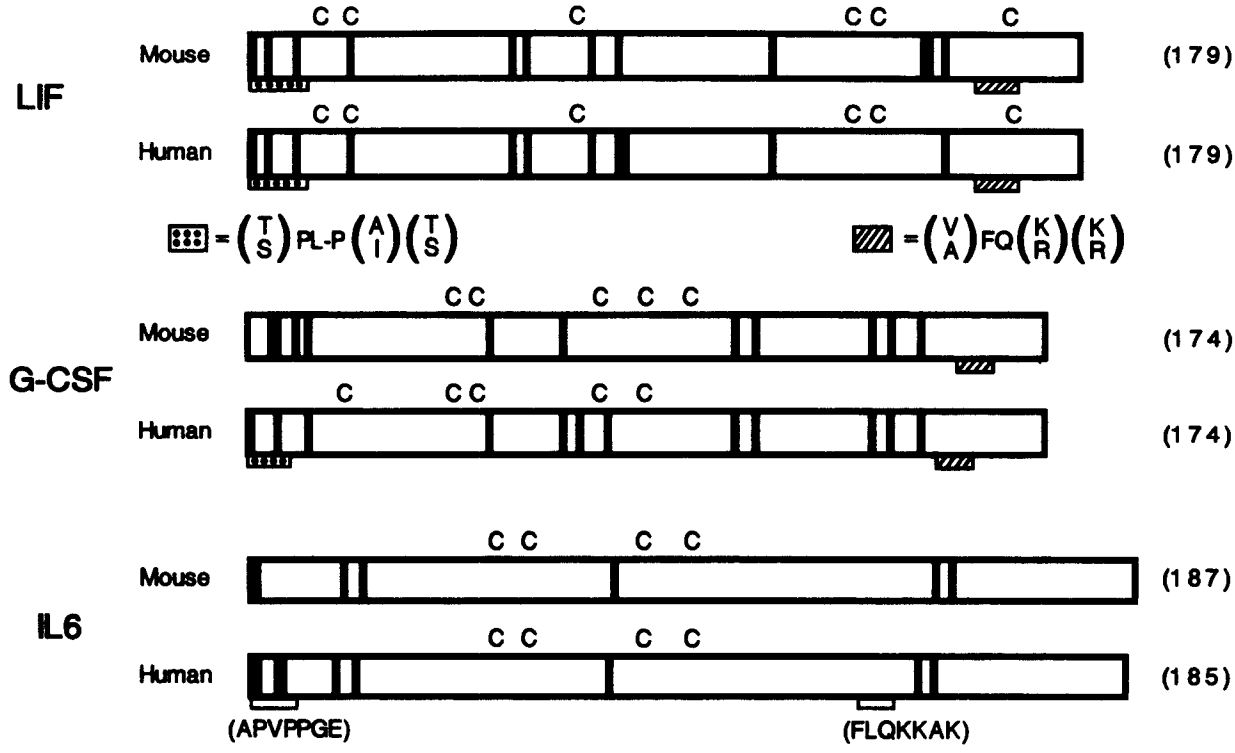

Fig. 1. Comparison of LIF, G-CSF and IL-6 polypeptides. Proline residues are marked by *vertical bars* and cysteine residues as *c*. Regions of homology are indicated by shaded boxes. (Based on reports in [10, 11, 13, 20, 22, 23]

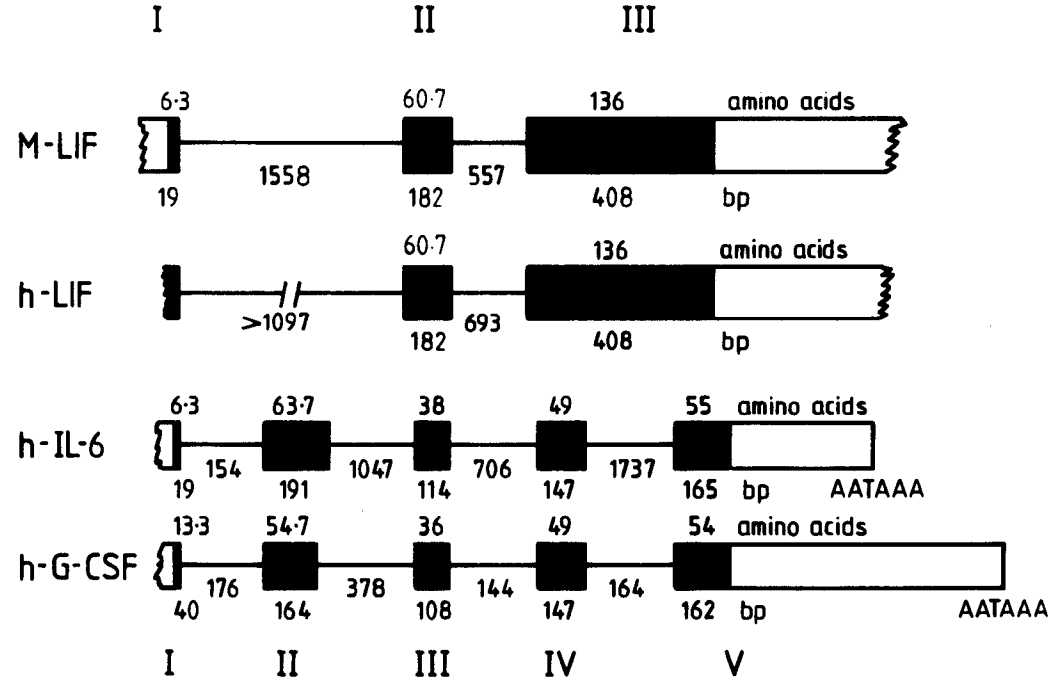

Fig. 2. Comparison of the gene organization between IL-6, G-CSF and LIF. *Boxes* represent exons. The coding and noncoding regions are shown by *closed* and *open boxes*, respectively. The *numbers above and below the boxes* indicate the numbers of amino acids of exons and those of nucleotides of exons for coding region, respectively. The *numbers below the lines* show the length of introns. (After the scheme presented in [24] and the data of [10] and [23]. Murine LIF gene organization: Gearing, King and Gough, Unpublished work)

same length as that of human IL-6 (6.3 codons), the lengths of the other exons are dissimilar. These data indicate that there is no reason to suggest that the three genes are related. However, it is interesting to note that the sum of the codons in the last three exons of the human G-CSF and IL-6 genes (139 and 142 codons, respectively) is approximately the same as the number of codons in the last exon of the LIF genes (136 codons). This suggests that if these genes are ancestrally related, then the LIF gene could have arisen by exon fusion of three exons into one, or that the G-CSF and IL-6 genes could have been derived by two introns inserting into a common larger last exon.

With the reasonable assumption that IL-6 is the molecule detected earlier as a plasmacytoma growth factor [25, 26], IL-6, G-CSF and LIF share two features in common: (a) when mice are injected with endotoxin, levels of each factor rise sharply in the serum within 1–3 h, and (b) all three molecules are produced in vitro by macrophages after priming with endotoxin [25–28] (D. Metcalf, personal communication), and G-CSF and LIF mRNAs are induced in macrophage lines following stimulation with endotoxin (N. Gough, personal communication). This suggests some intended biological coordination in their actions in vivo and raises the possibility of common transcriptional activation pathways.

D. Conclusions

From its primary protein sequences and its genetic organization, it can be concluded that LIF is a new structural entity which is probably not related to either G-CSF or IL-6, although the three molecules might in some ways be functionally related. At present, LIF appears unique in that it is not apparently a proliferative stimulus for either normal or leukaemic progenitor cells and it specifically inhibits colony formation and causes terminal differentiation of M1 leukaemic cells, this contrasts with G-CSF and IL-6, which can provide both a differentiative stimulus to M1 cells and a proliferative stimulus to normal cells.

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Note added in proof:
Since this manuscript was submitted, LIF has been shown to be able to suppress the human leukemic cell lines HL60 and U937 when used in combination with GM-CSF or G-CSF [29]. Somewhat surprisingly, and by contrast to its differentiation-inducing activity on M1 leukaemic cells, LIF also has a powerful differentiation-inhibiting activity on embryonic stem [ES] cells [30, 31] and its use will aid in their propagation in vitro. In a further twist, COS cell conditioned-media containing LIF can also apparently act as a growth stimulus for the murine haemopoietic cell line DA-1a [32] so its postulated role as a differentiation-only factor may have to be reconsidered.

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