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Partial Characterization of Murine Haematopoietic Cell Growth Factor mRNA

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Specific regulatory molecules are required for the growth and development of haematopoietic cells in vitro. Some of these molecular species appear to have a broad specificity, being able to promote the proliferation and differentiation of multipotential cells, as well as megakaryocytic, erythroid and granulocytic progenitor cells. Haematopoietic cell growth factor (HCGF) is a glycoprotein of molecular weight 28 500 which is produced constitutively by WEHI-3b myelomonocytic leukaemic cells and stimulated lymphoid cell populations in vitro [1]. Comparative studies [2] have shown that HCGF may share most, if not all, of the biochemical and biological properties of factors described variously as interleukin-3 (IL3) [3], burst-promoting activity (BPA) [4], mast cell growth factor (MCGF) [5], multicolony-stimulating factor (multi-CSF) [1], P cell-stimulating factor [6], histamine-producing cell-stimulating factor [7] and Thy-1-inducing activity [8].

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Fig. 1. Published murine 1L3 sequence [3]. Coding sequence is indicated by *brackets*. Oligonucleotide probes, complementary to mRNA sequences, were synthesized for those areas in boxes. Probe (121–138) (shown below) contains codon degeneracies and corresponds to the NH_2-terminus sequence of mature IL3. Such degeneracies were included to increase the probability of detecting homologous human sequences. In subsequent RNA blot experiments, an equimolor mixture of these probes was labelled using T4 polynucleotide kinase and [^{32}P]-labelled γ-ATP

5′GAC.ACA.CAC.CGA.TTA.ACAA3′

T C T C T C
G G G
T T T

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          10         20         30         40         50         60
  1 AACCCTTGGA GGACCAGAAC GAGAACAATG GTTCTTGCCA GCTCTACCAC CAGCATCCAC
 61 ACCATGCTGC TCCTGCTCAT GCTCTTCCAC CTGGGACTCC AAGCTTCAAT CAGTGGCCGG
121 GATACCCACC TTTAACCAGA ACGTTGAATT GCAGCTCTAT TGTCAAGGAG ATTATAGGGA
181 AGCTCCCAGA ACCTGAACTC AAAACTGATG ATGAAGGACC CTCTCTGAGG AATAAGAGCT
241 TTCGGAGAGT AAACCTGTCC AAATTCGTGG AAAGCCAAGG AGAAGTGGAT CCTGAGGACA

          310        320        330        340        350        360
301 GATACGTTAT CAAGTCCAAT CTTCAGAAAC TTAACTGTTG CCTGCCTACA TCTGCGAATG
361 ACTCTGCGCT GCCAGGGGTC TTCATTCGAG ATCTGGATGA CTTTCGGAAG AAACTGAGAT
421 TCTACATGGT CCACCTTAAC GATCTGGAGA CAGTGCTAAC CTCTAGACCA CCTCAGCCCG
481 CATCTGGCTC CGTCTCTCCT AACCGTGGAA CCGTGGAATG TTAAAACAGC AGGCAGAGCA
541 CCTAAAGTCT GAATGTTCCT CATGGCCCAT GGTCAAAAGG ATTTTACATT CCTTTATGCC

          610        620
601 ATCAAATGTC TTATCAATTT ATCTA
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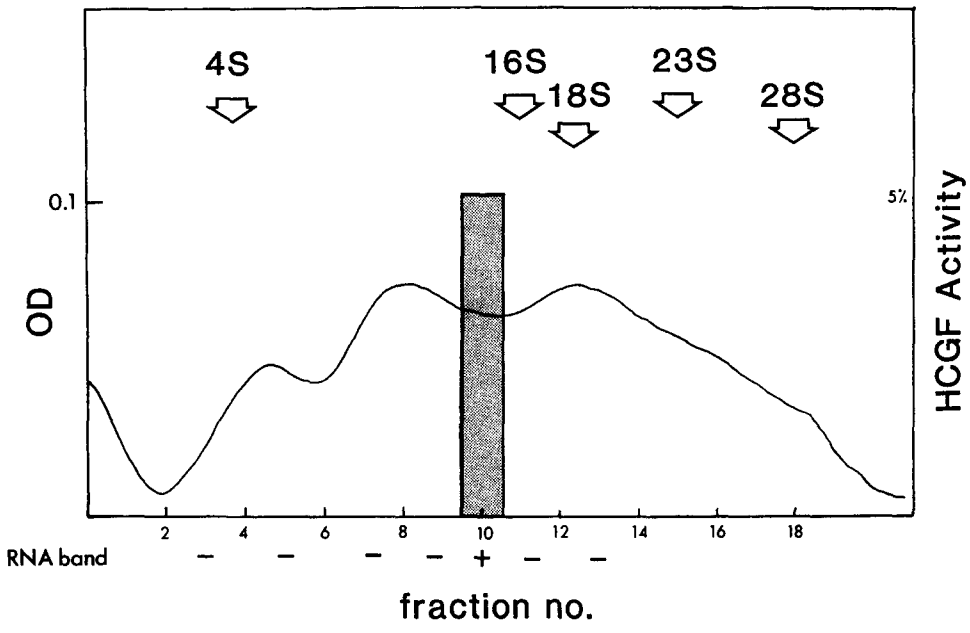

Fig. 2. Sucrose density gradient of WEHI-3b mRNA showing fractions that give HCGF activity when translated in *Xenopus* oocytes and hybridized to IL3 probes. Total RNA was prepared by cell lysis in 10 m*M* ribonucleoside–vanadyl complexes as RNase inhibitor and phenol extraction. Further purification involved oligo-dT-cellulose chromatography. RNA was applied to exponential 15%–50% sucrose gradients and centrifuged at 37 000 rpm for 18 h at 15 °C (SW41). The gradients were fractionated using an Isco UV monitor. Following ethanol precipitation, RNA from each fraction was redissolved in water (0.1 mg/ml) prior to microinjection into *Xenopus* oocytes. Secreted HCGF activity was measured using the factor-dependent cell (FDC-P) assay and shown in the *hatched area.* RNA from each fraction was also treated with glyoxal, separated by electrophoresis on 0.8% agarose gels and transferred to nitrocellulose. RNA blots were prehybridized in 6×SSC, 0.1% SDS, 5×Denharts for 48 h at 45 °C before the addition of labeled probes for a further 48 h at 45 °C. The filter was washed in 6×SSC for 10 min at 25 °C and 20 min at 45 °C

Recently cDNA sequences for IL3 [3] and MCGF [5] have been shown to be identical (Fig. 1).

Prior to cloning and expressing the cDNA gene for HCGF in *Escherichia coli,* we have partially characterized the mRNA which codes for HCGF by translation of mRNA size classes in *Xenopus* oocytes and RNA blot analysis using oligonucleotide probes to IL3 gene sequences (Fig. 2). We have also attempted to identify putative human IL3 sequences in mRNA isolated from mitogen-stimulated, lymphocyte-enriched buffy coat cells (Fig. 3).

We concluded that a 15 *S* mRNA species isolated from WEHI-3b cells is capable of coding for HCGF. Identical fractions contain a mRNA, 1100 nucleotides long, which hybridizes with murine IL3 oligonucleotide probes. These results suggest that the cDNA gene for HCGF is identical to those of IL3 and MCGF. Biological results indicate that IL3 and HCGF are the same. Recombinant IL3 (monkey COS cells/SV40 vector) displays almost identical biological behaviour to purified HCGF (T. M. Dexter, unpublished work).

In experiments designed to detect human IL3 sequences, oligonucleotide probes to the NH_2 terminus protein sequence of murine IL3 failed to hybridize to human mRNA isolated from mitogen-stimulated lymphocytes. Using the same experimental conditions and oligonucleotide probes of the same size and composition, IFN-γ and IL2 messenger RNA species of the correct size were observed. The failure of these experiments may be due to the lack of homologous sequences or its low abundance in human mRNA prepared by this mitogen regime. Cloning of the complete HCGF gene may facilitate the detection of human sequences on a genomic level.

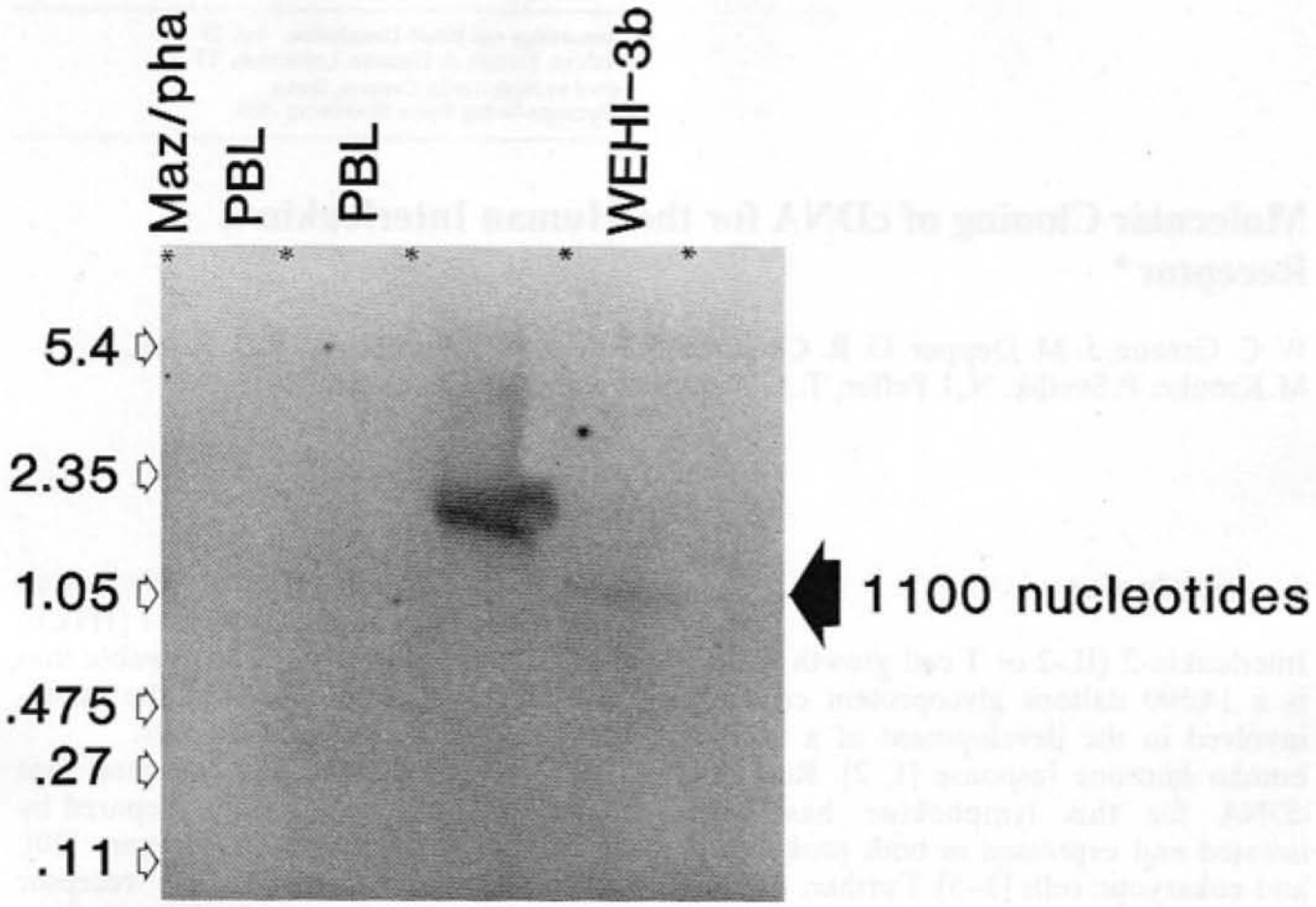

Fig. 3. RNA blot analysis of mRNA from: WEHI-3 cells; human buffy coat cells; and Mazerein/PHA-stimulated human buffy coat cells. Human mononuclear cells were isolated from buffy coats by Ficoll–Hypaque gradients. Cultures containing 5×10^6 mononuclear cells per millilitre in RPMI 1640 medium containing 1% fetal calf serum were treated with Mazerein (50 ng/ml) for 3 h at 37 °C; PHA was then added at a concentration of 10 μg/ml. The mean IFN-γ titre per induction was 3.9 $\log_{10}$ IU/ml after 24 h at 37 °C

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