within or adjacent to the centromeric heterochromatin (49.9 percent), in bands p22–p32 (14.6 percent), q31–32 (14.3 percent), and p36 (8.2 percent) (*16*). Whether this preponderance of chromosome 1 abnormalities is the result of clonal evolution of malignant cells, perhaps related to an unidentified proliferative advantage for the cell, or is part of the initial event in neoplastic transformation remains to be determined. Furthermore, the relation of these chromosomal abnormalities to the oncogenes located on chromosome 1 is unknown. The large physical distance between the mapped oncogenes and the common chromosome 1 lesions, especially 1q31–32, suggest that if these abnormalities play a role in the malignant transformation of the tumors in which they are found, additional oncogenes may also be mapped to this chromosome.

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References and Notes

1. R. Taub, I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982); J. Erikson, A. ar-Rushdi, H. L. Drwinga, P. C. Nowell, C. M. Croce, *ibid.* **80**, 820 (1983); J. Erikson, J. Finan, P. C. Nowell, C. M. Croce, *ibid.* **79**, 5611 (1982); J. M. Adams, S. Gerondakis, E. Webb, L. M. Corcoran, S. Cory, *ibid.* **80**, 1982 (1983); R. Dalla-Favera, S. Martinotti, R. C. Gallo, J. Erikson, C. M. Croce, *Science* **219**, 963 (1983).
2. G. Manolov and Y. Manolova, *Nature (London)* **237**, 33 (1972); L. Zech, U. Haglund, K. Nilsson, G. Klein, *Int. J. Cancer* **17**, 47 (1976); Y. Manolova, G. Manolov, J. Kieler, A. Levan, G. Klein, *Hereditas* **90**, 5 (1979); I. Miyoshi, S. Hiraki, I. Kimura, K. Miyamoto, J. Sato, *Experientia* **35**, 742 (1979); A. Bernheim, R. Berger, G. Lenoir, *Cancer Genet. Cytogenet.* **3**, 307 (1981); F. Mitelman, *Adv. Cancer Res.* **34**, 141 (1981); G. M. Lenoir, J. L. Preud'homme, A. Bernheim, R. Berger, *Nature (London)* **298**, 474 (1982); H. Van Den Berghe, C. Parlior, S. Gosseye, V. Englebienne, G. Cornu, G. Sokal, *Cancer Genet. Cytogenet.* **1**, 9 (1979); R. Berger *et al.*, *Hum. Genet.* **53**, 111 (1979).
3. R. Dalla-Favera, M. Bregni, J. Erikson, D. Patterson, R. C. Gallo, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982); B. G. Neel *et al.*, *ibid.*, p. 7842.
4. G. Klein, *Nature (London)* **294**, 313 (1981); J. D. Rowley, *Science* **216**, 749 (1982).
5. A. Diamond, G. M. Cooper, J. Ritz, M.-A. Lane, *Nature (London)* **305**, 112 (1983).
6. G. Goubin, D. S. Goldman, J. Luce, P. E. Neiman, G. M. Cooper, *ibid.* **302**, 114 (1983).
7. G. M. Cooper and P. E. Neiman, *ibid.* **292**, 857 (1981).
8. M. Harper and G. Saunders, *Chromosoma* **83**, 431 (1981).
9. I. R. Kirsch, C. C. Morton, K. Nakahara, P. Leder, *Science* **216**, 301 (1982).
10. D. G. Harnden, J. E. Lindsten, K. Buckton, H. P. Klinger, Eds., *Cytogenet. Cell Genet.* **31**, 5 (1981).
11. J. Ryan, P. E. Barker, K. Shimizu, M. Wigler, F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4460 (1983).
12. Y. E. Miller, C. Jones, C. Scoggin, H. Morse, P. Seligman, *Am. J. Hum. Genet.* **35**, 573 (1983).
13. G. D. Plowman, J. D. Brown, C. A. Enns, J. Schröder, B. Nikinmaa, H. H. Sussman, K. E. Hellström, I. Hellström, *Nature (London)* **303**, 79 (1983).
14. A. A. Sandberg, *Hum. Pathol.* **12**, 531 (1981); R. Abe, C. K. Tebbi, H. Yasuda, A. A. Sandberg, *Cancer Genet. Cytogenet.* **7**, 185 (1982); H. D. Solomons, B. Mendelow, A. Levinstein, A. E. Johnson, R. Bernstein, *Am. J. Hematol.* **14**, 297 (1983).
15. A. A. Sandberg, *The Chromosomes in Human Cancer and Leukemia* (Elsevier, New York, 1981), pp. 567–575.
16. V. Brito-Babapulle and N. B. Atkin, *Cancer Genet. Cytogenet.* **4**, 215 (1981).
17. C.C.M., R.A.T., and A.D. are supported by fellowships from the Damon Runyon–Walter Winchell Cancer Fund Fellowship. This investigation was supported in part by a faculty research award to G.M.C. from the American Cancer Society and NIH research grants GM-17088, CA-21082, and CA-33108. We also acknowledge the support of grants from the American Business Cancer Research Foundation and E. I. duPont de Nemours & Co. We thank T. Broderick for preparation of the manuscript.

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Gene for T-Cell Growth Factor: Location on Human Chromosome 4q and Feline Chromosome B1

Abstract. *T-cell growth factor (TCGF) or interleukin-2 (IL-2), an immunoregulatory lymphokine, is produced by lectin- or antigen-activated mature T lymphocytes and in a constitutive manner by certain T-cell lymphoma cell lines. By means of a molecular clone of human TCGF and DNA extracted from a panel of somatic cell hybrids (rodent cells × normal human lymphocytes), the TCGF structural gene was identified on human chromosome 4. In situ hybridization of the TCGF clone to human chromosomes resulted in significant labeling of the midportion of the long arm of chromosome 4, indicating that the TCGF gene was located at band q26–28. Genomic DNA from a panel of hybrids prepared with HUT-102 B2 cells was examined with the same molecular clone. In this clone of cells, which produces human T-cell leukemia virus, the TCGF gene was also located on chromosome 4 and was apparently not rearranged. The homologous TCGF locus in the domestic cat was assigned to chromosome B1 by using a somatic cell hybrid panel that segregates cat chromosomes. Linkage studies as well as high-resolution G-trypsin banding indicate that this feline chromosome is partially homologous to human chromosome 4.*

T-cell growth factor (TCGF) was originally discovered as a component of conditioned medium from lectin-stimulated primary cultures of human lymphoid cells that permitted long-term proliferation in vitro (*1, 2*). Exploitation of this discovery led to the cloning of T-cell populations of defined function and specificity (*3*) and the use of TCGF to establish cell lines from certain mature T-cell tumors (*4*). These cells respond to TCGF without prior activation with lectin or antigen, apparently because of constitutive expression of the TCGF receptor (*5*). From these cell lines the retrovirus human T-cell leukemia virus (HTLV) was isolated (*6*). TCGF-dependent lymphocyte cultures of normal or leukemic individuals are invariably mature T cells as determined by the expression of an array of T-cell markers and the lack of any B cell or immature lymphoid determinants (*1*). Although normal lymphocyte cultures require the continuous presence of TCGF for growth, many of the HTLV-infected cell lines gradually become independent of exogenous TCGF (*2*), suggesting that they either produce their own TCGF or by-pass the normal TCGF–TCGF receptor system required for proliferation.

Homogeneously purified human TCGF is a single polypeptide with a molecular weight of 15,000 (*7*). Recently, almost identical molecular clones of the structural gene for human TCGF were derived from complementary DNA (cDNA) libraries of a leukemic T-cell line (Jurkat) (*8*) and from normal peripheral blood lymphocytes (*9*). Use of cloned TCGF sequences as probes suggested that human TCGF is encoded by a single cellular gene containing introns. We have used a molecular TCGF clone to map the gene in the normal human genome and in the genome of the HUT-102 B2 lymphoma cell line from which the original isolate of HTLV was obtained. HUT-102 B2 has become TCGF-independent and produces low levels of TCGF messenger RNA (mRNA) and biologically active TCGF (*2, 9*).

TCGF was mapped in normal cells with use of a panel of 43 somatic cell hybrids (rodent cells × normal human lymphocytes) (*10*). The human chromo-

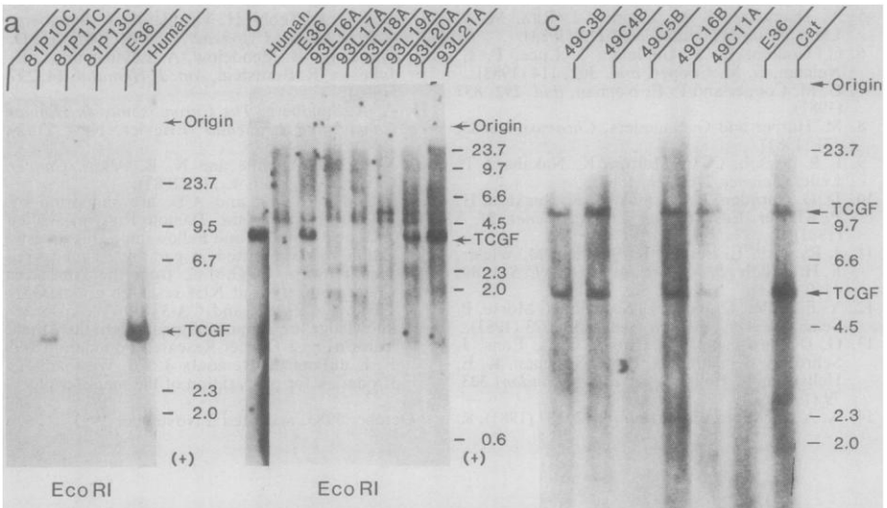

Fig. 1. Hybridization of a molecularly cloned TCGF probe to Eco RI digests of: (a) normal human lymphocytes × E36 Chinese hamster somatic cell hybrids; (b) HUT-102 B2 × E36 Chinese hamster cell hybrids; and (c) normal feline lymphocytes × E36 Chinese hamster cell hybrids. Restriction endonuclease-digested genomic DNA was subjected to electrophoresis on 0.7 percent agarose gels, transferred to nitrocellulose filters (*14*), and hybridized with a ^{32}P-labeled TCGF cDNA probe (*9*) in a solution containing 50 percent deionized formamide, 0.01 percent bovine serum albumin, 0.01 percent Ficoll, and 0.01 percent polyvinylpyrrolidone, a threefold (3×) concentration of SSC (1× SSC is 0.15*M* sodium chloride plus 0.015*N* sodium citrate), 20 m*M* sodium phosphate buffer, *p*H 6.5, and 10 percent dextran sulfate. After hybridization at 42°C for 24 to 48 hours, the filters were rinsed in 2× SSC, washed for 60 minutes in three changes of 0.2× SSC, and 0.1 percent sodium dodecyl sulfate at 50°C, rinsed in 0.2× SSC, and exposed to x-ray film for 7 to 10 days at −70°C.

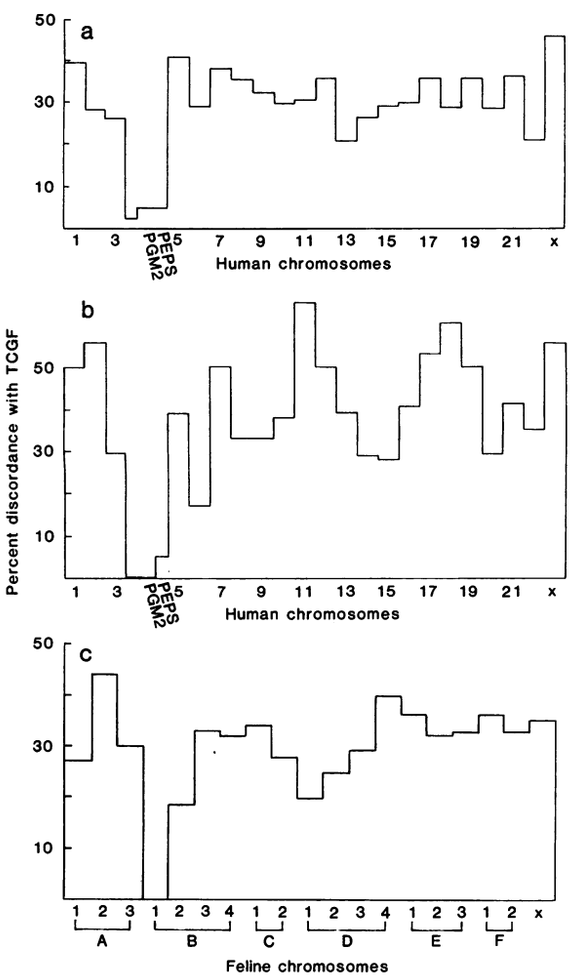

Fig. 2. Concordance of 23 human or 19 feline chromosomes and *TCGF* gene in three different panels of somatic cell hybrids (see text). Chromosome scores represent the consensus result of karyotypic and isoenzyme scores. Thirty-six isoenzyme systems diagnostic for each human chromosome were run on each human × rodent cell hybrid (*13*). Thirty-three isoenzyme systems diagnostic for feline chromosomes were run on each cat × rodent cell hybrid (*11, 13*). The details of isoenzyme analysis of human and feline cell hybrids has been described previously (*13*). The percentage discordance is presented for each chromosome with TCGF for: (a) 43 normal human lymphocyte × rodent somatic cell hybrids; (b) 18 HUT-102 B2 × rodent somatic cell hybrids; and (c) 37 cat × rodent somatic cell hybrids.

some constitution was monitored by G-trypsin banding (*10, 11*), G-11 chromosome staining (*12*), and electrophoretic resolution of up to 36 isozyme markers previously mapped to each of the 23 human chromosomes (*13*). The TCGF gene was visualized as a 3.5 kilobase (kb) doublet-fragment in human DNA after digestion with Eco RI (Fig. 1a) and Southern hybridization analysis (*14*) with a nick-translated probe derived from a 1.1-kb molecular clone that includes the human TCGF locus (*9*). Under the stringent conditions used (see legend to Fig. 1), Eco RI digests of the rodent cell DNA produced no hybridization in this region of the filter. Thus, by Eco RI digestion of the hybrid panel (Fig. 1a), it was possible to determine which hybrids retained the human TCGF gene and the chromosome on which it resided. The presence of the TCGF fragment was 98 percent concordant with human chromosome 4 (HSA4) and its included isozyme loci phosphoglucomutase 2 (*PGM2*) and peptidase S (*PEPS*) (Fig. 2a). The single discordant hybrid clone, 80H2C, had the genotype *HSA4*$^-$, *PGM2*$^-$; *PEPS*$^-$; *TCGF*$^+$. Because this hybrid did not contain any obvious rearranged human chromosomes, it may have contained a small transposition of HSA4 which was not apparent in the G-11 and G-trypsin analysis. Each of the remaining 22 human chromosomes showed high discordance (22 to 46 percent; Fig. 2a) with TCGF permitting the assignment of TCGF to human chromosome 4.

In situ hybridization of the TCGF cDNA clone to normal human chromosome preparations confirmed the assignment of TCGF to HSA4 and further identified the gene on the long arm. A nick-translated ^{3}H-labeled TCGF probe was hybridized at different concentrations between 10 and 100 ng/ml and then exposed autoradiographically for 4 to 27 days (*15*). In an analysis of 60 mitotic spreads, 33 percent of the metaphase spreads that could be scored exhibited label on the midportion of HSA4q. These labeled sites, each consisting of one to three grains, represented 20 percent (21 out of 107) of all labeled sites distributed throughout the 60 metaphase spreads. Compilation of grain positions from a large number (N = 47) of labeled No. 4 chromosomes indicated significant clustering of grains on segment q26–28 (65 percent) (Fig. 3a). These results are illustrated by the representative metaphase cell in Fig. 3b. Thus, on the basis of this significant labeling of a small segment on the long arm of chromosome 4, we concluded that the TCGF gene was on HSA4q26–28.

The dependence of certain HTLV-producing human cell lymphoma lines on TCGF for proliferation in vitro prompted us to examine these and other human cells for the molecular and chromosomal integrity of the TCGF locus. Restriction enzyme digests (with six enzymes) of nine human cell lines, including the HTLV-infected lines HUT-102 (*6*) and MJ (*16*), all expressed the same patterns after Southern blot analysis with the 1.1-kb TCGF molecular probe (*17*). We observed no intraspecific human polymorphisms or any HTLV- or tumor-associated perturbations of the TCGF locus. To examine the chromosomal position of TCGF in an HTLV-associated tumor, we prepared and characterized a panel of 18 somatic cell hybrids between E36 Chinese hamster cells and HTLV-producing human lymphoma HUT-102 B2 with G-11 stain and isozyme markers as described above. TCGF was 100 percent concordant with HSA4 and the associated isozyme markers, *PGM2* and *PEPS*, and appreciably discordant (17 to 60 percent) with the remaining human chromosomes (Figs. 1b and 2b). These results confirm the assignment of *TCGF* to HSA4 and are consistent with the absence of chromosomal or molecular rearrangement of the TCGF locus in the etiology of the HUT-102 lymphoma.

A striking conservation of linkage homology between the genetic maps of the domestic cat and man was recently demonstrated (*11, 18*). When the linkage map of 31 homologous biochemical loci in both species was compared, only a few exceptions to linkage conservation were observed. We used the human TCGF clone and a panel of 37 cat × rodent somatic cell hybrids to map the feline chromosomal homolog of *TCGF*. The panel was expanded and analyzed with human TCGF by the same strategies that we used for the human analysis. The feline TCGF gene was visualized as a pair of fragments of 5.2 and 9.7 kb after digestion of cellular DNA with Eco RI and Southern blot analysis with the 1.1-kb TCGF clone being used as a probe (Fig. 1c). Homologous DNA segments from rodents did not appear in this region of the gels. The two fragments from cat DNA were precisely concordant in 37 hybrids. The simplest interpretation of this observation is that an Eco RI site exists within the feline DNA sequence homologous to the TCGF probe.

The presence of feline TCGF was 100 percent concordant with feline chromosome B1 and highly discordant (30 to 60 percent) with the other 18 feline chromosomes (Fig. 2c), thereby permitting assignment of the feline TCGF gene to feline chromosome B1 (FCA B1). FCA B1 has previously been implicated as homologous to human chromosome 4 because both chromosomes contain the structural gene for the peptidase-S (*PEPS*) (*11, 18*). To extend this analysis, we sought to examine the possibility of cytological (G-banding) homology between these chromosomes. In Fig. 3c we show high-resolution banding of the feline chromosome B1, and of human chromosome 4 at a similar level of extension. The centromere proximal regions of the long arms of both chromosomes (FCA B1q and HSA4q12–24) displayed nearly identical banding. It is important to consider that the long arms of both human 4q and feline B1q are believed to be ancestral in that they do not contain the chromosome rearrangements that occurred after the divergence of the primate and carnivore orders (*18*). Because of the linkage cytological homologies reported here between the two chromosome arms, we propose that the proximal portions of HSA4q and FCA B1q be added to the five previously defined homologous chromosome segments between the primates and carnivores (*18*).

Our results indicate that human TCGF is encoded by a single locus on human chromosome 4q26–28. The only other lymphokine loci mapped to date in man are those of the interferon family: interferon, leukocyte type, HSA9 (*19*), and interferon, immune type, HSA12 (*20*). Other growth factors have not been chromosomally assigned, although a DNA sequence of human platelet–derived growth factor (PDGF) has recently been shown to be 90 percent homologous to the retroviral oncogene *sis* (*21*), which has been mapped to chromosome 22 (*22*). Presumably, the human homolog of *sis* codes for at least part of PDGF. Another oncogene, c-*raf* (*23*), has two homologous sequences in humans, one of which, c-*raf*-2, has been mapped to human chromosome 4 (*24*). The TCGF locus and c-*raf*-2 do not appear to be identical for two reasons. First, c-*raf*-2 is an apparent pseudogene which has termination signals in each reading frame (*25*). Second, the nucleotide sequences of *TCGF* (*14, 15*) and c-*raf*-2 (*25*) have been determined. A computer-assisted comparison of the two sequences using the ALIGN program (*26*) did not reveal any significant homology. In addition, c-*raf*-2 and *TCGF* are probably not closely linked since at least one of our hybrids, 80H9AC, has the genotype *HSA4*$^-$, *PGM2*$^-$, *PEPS*$^-$, *raf*-2$^+$, and *TCGF*$^-$. This hybrid has an unidentifiable rearranged human chromosome which apparently contains the *raf*-2 segment and none of the other HSA4 loci, including TCGF. The additional 15 human onco-

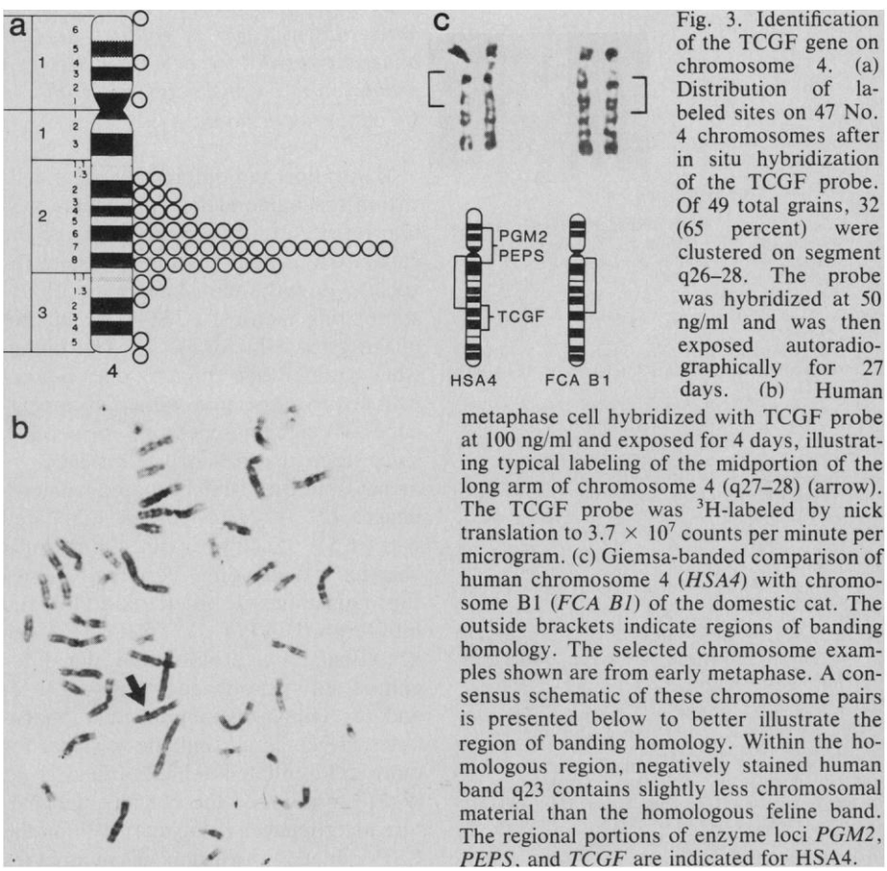

Fig. 3. Identification of the TCGF gene on chromosome 4. (a) Distribution of labeled sites on 47 No. 4 chromosomes after in situ hybridization of the TCGF probe. Of 49 total grains, 32 (65 percent) were clustered on segment q26–28. The probe was hybridized at 50 ng/ml and was then exposed autoradiographically for 27 days. (b) Human metaphase cell hybridized with TCGF probe at 100 ng/ml and exposed for 4 days, illustrating typical labeling of the midportion of the long arm of chromosome 4 (q27–28) (arrow). The TCGF probe was ^{3}H-labeled by nick translation to 3.7 × 10^7 counts per minute per microgram. (c) Giemsa-banded comparison of human chromosome 4 (*HSA4*) with chromosome B1 (*FCA B1*) of the domestic cat. The outside brackets indicate regions of banding homology. The selected chromosome examples shown are from early metaphase. A consensus schematic of these chromosome pairs is presented below to better illustrate the region of banding homology. Within the homologous region, negatively stained human band q23 contains slightly less chromosomal material than the homologous feline band. The regional portions of enzyme loci *PGM2*, *PEPS*, and *TCGF* are indicated for HSA4.

gene loci mapped to date are dispersed on other human chromosomes (*27*).

That growth factors may play a role in the onset of neoplasia has been an attractive hypothesis for some time. The near identity of a gene sequence of *PDGF* and v-*sis* has added credence to this hypothesis (*21*). A characteristic translocation involving chromosome 4 (q21) and chromosome 11 has been observed in some cases of acute leukemia (*28*). However, recent phenotypic studies suggesting that the t(4;11)-associated acute leukemia represents a proliferation of myeloid progenitor cells (*29*) as well as the identification of the TCGF gene on q26–28 make it difficult to implicate TCGF in the etiology of this disease. Nonetheless, the occurrence of *TCGF* on chromosome 4 adds an important aspect to the continuing genetic analysis of oncogenes, growth factors, and retroviruses that have been implicated as regulators of normal growth and development as well as in the etiology of neoplasia (*27*).

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References and Notes

1. D. A. Morgan, F. W. Ruscetti, R. C. Gallo, *Science* **193**, 1007 (1976); F. W. Ruscetti, D. A. Morgan, R. C. Gallo, *J. Immunol.* **119**, 131 (1977); S. Gillis, M. M. Ferm, W. Ou, K. A. Smith, *ibid.* **120**, 2027 (1978); F. W. Ruscetti, J. W. Mier, R. C. Gallo, *J. Supramol. Struct.* **13**, 229 (1980).
2. J. E. Gootenberg, F. W. Ruscetti, J. W. Mier, A. Gazdar, R. C. Gallo, *J. Exp. Med.* **154**, 1403 (1981).
3. S. Gillis and K. A. Smith, *Nature (London)* **268**, 154 (1977); S. Gillis, P. E. Baker, F. W. Ruscetti, K. A. Smith, *J. Exp. Med.* **148**, 1093 (1978); J. Watson, *ibid.* **150**, 1510 (1979).
4. B. J. Poiesz, F. W. Ruscetti, J. W. Mier, A. M. Woods, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6815 (1980); R. C. Gallo, B. J. Poiesz, F. W. Ruscetti, in *Modern Trends in Human Leukemia*, R. Neth, R. C. Gallo, T. Graft, K. Mannweiler, K. Winkler, Eds. (Springer-Verlag, Munich, 1981), vol. 5, pp. 502–514.
5. T. Waldmann *et al.*, *Clin. Res.* **31**, 547a (1983).
6. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
7. J. W. Mier and R. C. Gallo, *ibid.*, p. 6134.
8. T. Taniguchi, H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto, J. Hamuro, *Nature (London)* **302**, 305 (1983).
9. S. Clark *et al.*, in preparation.
10. S. J. O'Brien, W. G. Nash, J. L. Goodwin, D. R. Lowry, E. H. Chang, *Nature (London)* **302**, 839 (1983); S. J. O'Brien, T. I. Bonner, M. Cohen, C. O'Connell, W. G. Nash, *ibid.* **303**, 74 (1983).
11. S. J. O'Brien and W. G. Nash, *Science* **216**, 257 (1982).
12. M. Bobro and J. Cross, *Nature (London)* **251**, 77 (1974).
13. S. J. O'Brien, J. M. Simonson, M. E. Eichelberger, in *Techniques in Somatic Cell Genetics*, J. Shay, Ed. (Plenum, New York, 1982); T. B. Shows and P. J. McAlpine, *Cytogenet. Cell Genet.* **32**, 221 (1982); H. Harris and P. A. Hopkinson, in *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).
14. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
15. M. E. Harper, G. Franchini, J. Love, M. I. Simon, R. C. Gallo, F. Wong-Staal, *Nature (London)* **304**, 169 (1983); M. E. Harper, A. Ullrich, G. F. Saunders, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4458 (1981); M. E. Harper and G. F. Saunders, *Chromosoma* **83**, 431 (1981).
16. M. Popovic *et al.*, *Science* **219**, 856 (1983).
17. The human cells examined included: HeLa, BeWo, 143B, A673, JAR, WiDR, VA-2 [S. J. O'Brien *et al.*, *In Vitro* **16**, 119 (1980)], HUT-102 B2 p105 (*16*), and MJ (*16*). The restriction endonucleases used were: Eco RI, Pst I, Hind I, Xba I, Hind III, and Sst I. The patterns for all the cells with each restriction enzyme were identical.
18. W. G. Nash and S. J. O'Brien, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6631 (1982).
19. D. Owerbach *et al.*, *ibid.* **78**, 3123 (1981).
20. S. L. Naylor, A. Y. Sakaguchi, T. B. Shows, M. L. Law, D. Goeddel, P. W. Gray, *J. Exp. Med.* **157**, 1020 (1983).
21. R. F. Doolittle *et al.*, *Science* **221**, 275 (1983); M. D. Waterfield *et al.*, *Nature (London)* **304**, 35 (1983).
22. R. Dalla-Favera, R. C. Gallo, A. Giallongo, C. M. Croce, *Science* **218**, 686 (1982); D. C. Swan, O. W. McBride, K. C. Robbins, D. A. Keithley, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4691 (1982).
23. U. R. Rapp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4218 (1983).
24. T. I. Bonner, S. J. O'Brien, W. G. Nash, U. R. Rapp, C. C. Morton, P. Leder, *Science* **223**, 71 (1984).
25. G. Mark and U. R. Rapp, personal communication.
26. M. O. Dayhoff and W. C. Barker, *Atlas of Protein Sequence and Structure 1965* (National Biochemical Research Foundation, Washington, D.C., 1978).
27. J. D. Rowley, *Nature (London)* **301**, 290 (1983); see S. J. O'Brien, Ed., *Genetic Maps* [(Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1984), vol. 3], for complete reference.
28. M. Oshimura, A. I. Freeman, A. A. Sanberg, *Cancer (Brussels)* **40**, 1161 (1977); E. L. Prigogina *et al.*, *Hum. Genet.* **53**, 5 (1979); H. Van Den Berghe *et al.*, *ibid.* **46**, 173 (1979).
29. J. L. Parkin *et al.*, *Blood* **60**, 1321 (1982); M. Nagasaka *et al.*, *ibid.* **61**, 1174 (1983).
30. We thank J. Simonson, R. Bauer, M. Eichelberger, N. White, and J. Love for excellent technical assistance and R. Reeves for discussion and technical advice. We thank G. Mark for communication of the unpublished sequence of c-*raf*-2 and for assistance in the sequence comparisons.
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Endogenous Regulation of Macrophage Proliferative Expansion by Colony-Stimulating Factor–Induced Interferon

Abstract. *Stimulation of cultures of murine bone-marrow cells with specific macrophage growth factor (colony-stimulating factor 1) resulted in the production of type 1 interferon. Neutralization of this endogenous interferon by antiserum directed against interferons α and β resulted in a significant enhancement of mononuclear phagocyte proliferation from committed marrow precursors. The effect of the antiserum was lost in cultures depleted of adherent cells, an indication that an adherent regulatory cell (or cells) in the marrow limits mononuclear phagocyte proliferation by producing antiproliferative interferon in response to high levels of specific growth factor.*

Maturation and differentiation of cells within the mononuclear phagocyte system results in a progressive loss of the capacity to proliferate in response to the specific growth factor known as colony-stimulating factor 1 (CSF-1) or macrophage growth factor (*1, 2*). The diminished proliferative capacity does not appear to be associated with a disappearance of specific receptors for CSF-1, since their expression is retained by more mature, differentiated macrophages (*2, 3*). Macrophages stimulated with CSF display various functional changes (*4*), including secretion of series E protaglandins (*5*), interleukin 1 (*6*), and interferon (IFN) (*4, 7*). Thus CSF-1 can act either as a proliferative signal for committed macrophage precursors (*2, 8*) and for immature mononuclear phagocytes (*1*) or as a stimulatory signal for more differentiated cells. Kurland *et al.* (*9, 10*) introduced the concept that mature macrophages in the marrow limit the CSF-induced expansion of monocytes and macrophages to appropriate levels by reacting to CSF within the local environment and producing series E prostaglandins; these prostaglandins act as potent inhibitors of CSF-induced mononuclear phagocyte proliferation from committed precursors (*11*). Thus CSF-1 may function in the bone marrow to regulate mononuclear phagocyte generation in both a positive and negative fashion. Series E prostaglandins, however, are not the only inhibitory molecules produced by CSF-stimulated macrophages. Interferon is also produced (*4, 7*) and may act as an antagonist to CSF-induced proliferation (*12–15*). Therefore mononuclear phagocyte proliferation might also be regulated by the balance of CSF and IFN in the environment of committed precursors. We obtained results that supported this hypothesis; the proliferative response of murine marrow preparations treated with purified CSF-1 was enhanced in the presence of antiserum that effectively neutralized murine IFN's