286-bp JCV fragment functioned better in the sense orientation in fetal glial cells, but in the antisense orientation in CV-1 cells. This is in contrast to the SV40 and BKV enhancers, which are consistently more active in the sense orientation, regardless of cell type. This difference in orientation preference among cell types may relate to the JCV enhancer activity or to a difference in the activity of the two TATA boxes. It may be significant that the only cell type in which JCV grows well is also the cell type in which the sense orientation of the JCV enhancer is more active. This preference for the sense orientation of the JCV enhancer region in fetal glial cells could relate to the orientation of transcription of T antigen, which in turn is necessary for DNA replication.

The mechanism by which the JCV enhancer-promoter stimulates brain-specific transcription is currently unknown. In the case of viruses which have evolved from cellular sequences, it seems reasonable that the proteins which interact with viral regulatory sequences perform an analogous function for cellular genes. An 82-nucleotide element, the identifier sequence has been found within the introns of precursor RNA molecules isolated from rat brain that was not detected in other rat tissues (*25, 26*). We have compared the nucleotide sequence of the JCV 98-bp repeat with this 82-nucleotide rat brain specific sequence and found marked regions of homology [significance level of $10^{-8}$; (*27*)] (Fig. 2). This suggests the presence of a brain-specific transcription factor that recognizes both the JCV transcriptional regulatory region and the 82-nucleotide rat brain sequence. Whether these identifier sequences serve a regulatory role in the transcription of brain-specific genes remains to be determined.

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### References and Notes

1. B. L. Padgett, D. L. Walker, G. M. Zurhein, A. E. Hodach, S. M. Chow, *J. Infect. Dis.* **133**, 686 (1976).
2. D. V. Coleman *et al.*, *ibid.* **142**, 1 (1980).
3. T. F. Hogan, E. C. Borden, J. A. McBain, B. L. Padgett, D. L. Walker, *Ann. Intern. Med.* **92**, 373 (1980).
4. B. L. Padgett, C. M. Rogers, D. L. Walker, *Infect. Immun.* **15**, 656 (1977).
5. A. M. Beckmann, K. V. Shah, B. L. Padgett, *ibid.* **38**, 774 (1982).
6. K. K. Takemoto, P. M. Howley, T. Miyamura, *J. Virol.* **30**, 384 (1979).
7. G. C. Fareed, K. K. Takemoto, M. A. Gimbrone, in *Microbiology*, D. Schlessinger, Ed. (American Society for Microbiology, Washington, D.C., 1978), p. 427.
8. C. Benoist and P. Chambon, *Nature (London)* **290**, 304 (1981).
9. P. Gruss, R. Dhar, G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 943 (1981).
10. N. Rosenthal, M. Kress, P. Gruss, G. Khoury, *Science* **222**, 749 (1983).
11. L. Laimons, P. Gruss, R. Pozzatti, G. Khoury, *J. Virol.* **49**, 183 (1984).
12. S. D. Gillies, S. L. Morrison, V. T. Oi, S. Tonegawa, *Cell* **33**, 717 (1983).
13. J. Banerji, L. Olson, W. Schaffner, *ibid.*, p. 729.
14. C. Queen and D. Galhmore, *ibid.*, p. 741.
15. D. Picard and W. Schaffner, *Nature (London)* **307**, 80 (1984).
16. M. Walker, T. Edlund, A. Boulet, W. Rutter, *ibid.* **306**, 557 (1983).
17. I. S. Chen, J. McLaughlin, D. W. Golde, *ibid.* **309**, 276 (1984).
18. J. Lenz, *et al.*, *ibid.* **308**, 467 (1984).
19. L. DesGroseillers, E. Rassart, P. Jolicoeur, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4203 (1983).
20. R. Frisque, *J. Virol.* **46**, 170, (1983).
21. Unpublished data.
22. F. Graham and A. Van der Erb, *Virology* **52**, 456 (1973).
23. C. Gorman, L. Moffat, B. Howard, *Mol. Cell. Biol.* **2**, 1044, (1982).
24. C. Gorman, G. Merlino, M. Willingham, I. Pastan, B. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
25. R. Milner, F. Bloom, C. Lai, R. Lerner, J. Sutcliffe, *ibid.* **81**, 713 (1984).
26. J. Sutcliffe, R. Milner, J. Gottesfeld, R. Lerner, *Nature (London)* **308**, 237 (1984).
27. W. B. Goad and M. I. Kanehisa, *Nucleic Acids Res.* **10**, 247 (1982).
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## Purified Human Granulocyte-Macrophage Colony-Stimulating Factor: Direct Action on Neutrophils

Abstract. *Neutrophil migration inhibition factor from T lymphocytes (NIF-T) is a lymphokine that acts to localize granulocytes. Medium conditioned by the Mo human T-lymphoblast cell line was used to purify NIF-T, a glycoprotein with a molecular weight of 22,000. The NIF-T was found to potently stimulate the growth of granulocyte and macrophage colonies from human bone marrow and colony formation by the KG-1 myeloid leukemia cell line. Thus a human lymphokine (NIF-T) that modulates the activities of mature neutrophilic granulocytes is also a colony-stimulating factor acting on precursors to induce growth and differentiation of new effector cells.*

Neutrophil migration inhibition factor from T lymphocytes (NIF-T) is a lymphokine produced by mitogen- and antigen-stimulated lymphocytes, the Mo T-lymphoblast cell line, and mature T cells transformed by human T-cell leukemia virus (HTLV) (*1, 2*). NIF-T is assayed by measuring its ability to inhibit migration of human peripheral blood neutrophils under agarose, and it is a potent activator of human neutrophils (*1, 3*). The Mo cells and lectin-stimulated lymphocytes also produce colony-stimulating factors (CSF) that act on myeloid precursor cells to give rise to colonies of differentiated granulocytes and mononuclear phagocytes in semisolid culture (*4*). To precisely define the biochemical and biological characteristics of lymphokines that act on early precursor cells (CSF) and mature neutrophils (NIF-T), highly purified preparations of these proteins are required. We recently developed a purification scheme that yields homogeneous erythroid-potentiating activity, a lymphokine regulating early erythropoiesis (*5*); the same approach was used to prepare highly purified NIF-T and CSF. Our results show that NIF-T and CSF activities reside in a single glycoprotein with apparent molecular weight of 22,000, demonstrating that a single human lymphokine acts on progenitor cells in the bone marrow and mature effector cells in the periphery. The identity of these molecules and activities has important implications for human physiology and the pathophysiology of inflammatory and immune disorders.

Ten liters of serum-free Mo-conditioned medium (the cells were grown in Iscove's modified Dulbecco's medium) were concentrated 30-fold with an Amicon hollow-fiber apparatus having an H1P10-8 filter. The concentrated protein was precipitated by ammonium sulfate at 80 percent saturation, dissolved in phosphate-buffered saline (PBS), dialyzed extensively, and clarified by centrifugation. The protein concentrate was applied to a lentil lectin Sepharose 4B column (2.6 by 6 cm; Pharmacia) equilibrated and washed with PBS. Glycoproteins were eluted with 0.5*M* α-methyl-D-mannoside in PBS and concentrated with a Centricon-10 microconcentrator (Amicon). The eluted glycoproteins were fractionated by size with an Ultrogel AcA 44 column (1.6 by 80 cm; LKB) equilibrated and eluted with PBS. Fractions of 2.5 ml were collected and assayed for NIF-T and CSF activities.

Colony-stimulating activity was found over a broad range in the Ultrogel fractions, most likely because of the presence of multiple CSF's in Mo-conditioned medium. However, a well-defined peak of NIF-T activity was found in a

pool of Ultrogel fractions corresponding to a molecular weight of approximately 30,000. A sample of the Ultrogel material containing NIF-T activity was concentrated and fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gel was sliced and the eluted proteins were assayed for NIF-T and CSF activities. A single peak was observed for both activities corresponding to an apparent molecular weight of 22,000 (Fig. 1).

The biological activities eluted from the gel shown in Fig. 1 did not correspond to a major peak of protein; therefore further purification was necessary. The pooled Ultrogel material was fractionated by reversed-phase high-performance liquid chromatography (HPLC) by using a Beckman instrument fitted with a Vydac C-18 column (10 by 250 mm) (*5*). Protein was eluted from the column with a gradient of acetonitrile containing 0.1 percent trifluoroacetic acid and elution was monitored by measuring absorbance at 220 nm (Fig. 2A). HPLC fractions A through I were evaporated to dryness, resuspended in 50 m*M* ammonium bicarbonate (*p*H 7.0), diluted in PBS containing 0.01 percent bovine serum albumin (BSA), and assayed for NIF-T and CSF activities. Figure 2B shows that both NIF-T and CSF activities were found in HPLC fractions H and I, which had eluted as a broad peak at approximately 48 percent acetonitrile.

The colony-stimulating activity of this highly purified preparation (HPLC fractions H and I in Fig. 2) was assayed on human bone marrow and the KG-1 human myeloid leukemia cell line. Figure 3A shows a typical colony formed by human bone marrow cells stimulated with HPLC-purified CSF. These colonies were stained for the chloracetate esterase found in neutrophilic granulocytes and the α-naphthyl acetate or butyrate esterase found in monocytes and macrophages. Both types of colonies were seen, consistent with the identification of this factor as a granulocyte-macrophage CSF previously shown to be a product of the Mo cells (*4*). Serial dilutions of HPLC fraction H stimulated

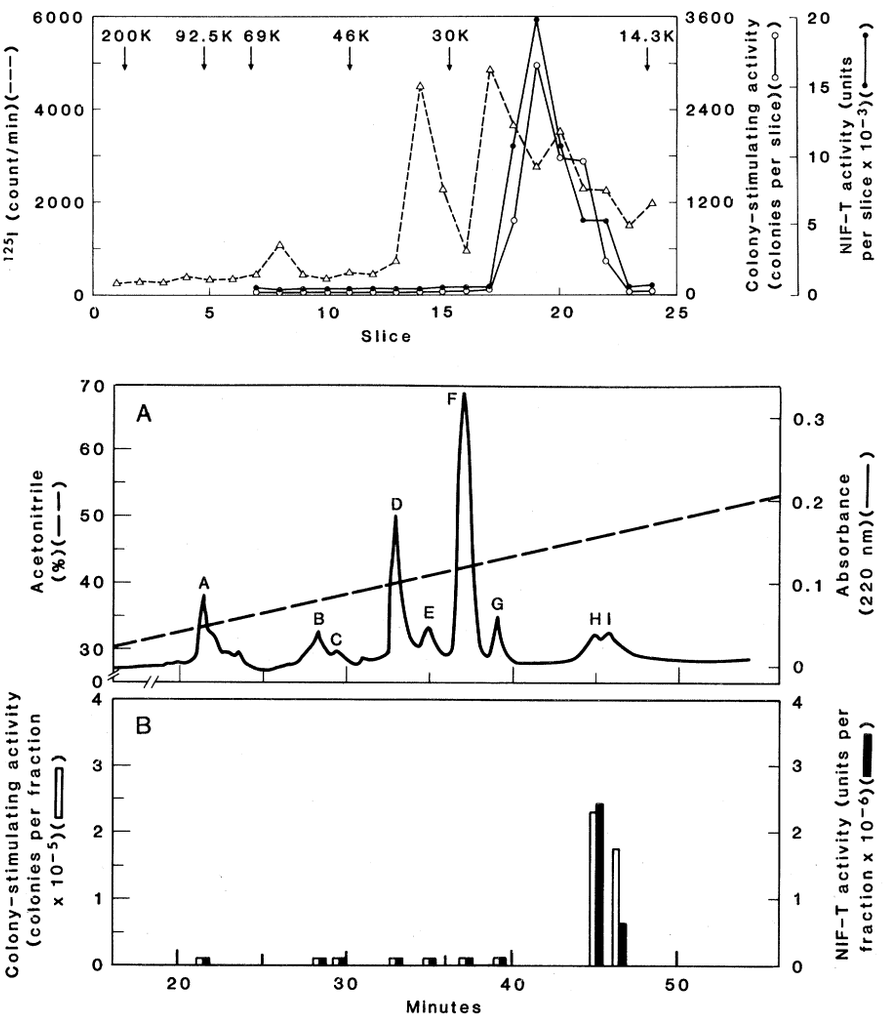


Fig. 1 (top). Apparent molecular weight of NIF-T and CSF from Ultrogel fractions. Protein from 10 liters of serum-free Mo-conditioned medium was concentrated and applied to a lentil lectin column; the lentil lectin eluate was fractionated with a Ultrogel AcA44 column (*5*). Two Ultrogel fractions at the trailing end of the peak of NIF-T activity were pooled and half the pool (approximately 2 ml) was dialyzed extensively against 20 m*M* ammonium bicarbonate, lyophilized, and resuspended in loading buffer containing 0.5 percent SDS without β-mercaptoethanol; the proteins were fractionated on a 10 percent polyacrylamide slab gel (*11*). A portion of the pooled fractions was iodinated by a modification of the chloramine T procedure (*12*) and the iodinated material (approximately 80,000 count/min) was added to the concentrated protein before electrophoresis. Slices of the running gel (3 mm) were eluted overnight into 0.25 ml of PBS containing 0.01 percent bovine serum albumin carrier protein. SDS was adsorbed by the addition of AG11A8 resin (Bio-Rad). Recovery of protein was determined on the basis of total counts per minute of $^{125}$I-labeled material in the slice relative to eluted radioactivity. Colony-stimulating activity was assayed with light-density nonadherent human bone marrow cells plated in microtiter wells at a density of $1.5 \times 10^4$ cells per 0.1 ml of agar-containing medium (*4*). Ten microliters of various dilutions of the protein eluted from gel slices were added to each well; each dilution was assayed in triplicate. Colonies of more than 50 cells were enumerated after 14 days. NIF-T activity was assayed as described elsewhere (*2*). Briefly, 4-μl dilutions of the proteins eluted from the gel slices were incubated with $2 \times 10^5$ human neutrophils at 37°C for 30 minutes; $2.5 \times 10^4$ neutrophils were then dispensed into each of four 1.5-mm wells in agarose plates. The plates were incubated overnight at 37°C in 5 percent $CO_2$ and then fixed with 95 percent ethanol. Migration diameters were measured with an ocular micrometer. One unit of NIF-T is the amount of mediator required to inhibit migration of $2.5 \times 10^4$ neutrophils 25 percent. The relative positions of molecular weight markers (molecular weight $\times 10^{-3}$) run in a separate lane (reduced with β-mercaptoethanol) are indicated. Fig. 2 (bottom). Reversed-phase HPLC elution profile. The material applied was prepared from 10 liters of conditioned medium as described in the legend of Fig. 1. Three Ultrogel fractions containing the peak NIF-T activity were pooled and half this material (approximately 3.5 ml) was applied to the Vydac C-18 column (10 by 250 mm) (*5*). The flow rate was 1.5 ml per minute, with a starting solvent of 10 percent acetonitrile and 0.1 percent trifluoroacetic acid in water. (A) The proteins were eluted with the following gradient: 10 to 30 percent acetonitrile over 10 minutes, then 30 to 60 percent over 60 minutes. Absorbance at 220 nm was monitored continuously. Fractions A through I were collected manually into siliconized glass tubes and evaporated to dryness. The protein was resuspended in 50 m*M* ammonium bicarbonate and serial dilutions for bioassay were made in PBS containing 0.01 percent bovine serum albumin. (B) Dilutions of the HPLC fractions were assayed for colony-stimulating activity on $2.2 \times 10^4$ human bone marrow cells per microtiter well and for NIF-T activity, as described in the legend to Fig. 1.

growth of bone marrow colonies and colonies from the myeloid KG-1 cell line in the same range of dilutions (Fig. 3B). The KG-1 cells were previously shown to respond to colony-stimulating activity (*6*). Since bone marrow cells were depleted of adherent accessory cells before assay and KG-1 cells were stimulated in the same range of concentrations, it is likely that this CSF acts directly on colony-forming cells and not indirectly through stimulation of accessory cells in the bone marrow.

The HPLC-purified material having both CSF and NIF-T activities was iodinated, fractionated by SDS-PAGE, and visualized by exposure of the dried gel to film. Two bands of labeled protein were seen: a broad band with a molecular weight of 26,000 to 28,000 and a band of 22,000 (Fig. 4A). Since it was possible that one protein corresponded to NIF-T activity and the other to CSF, the remaining unlabeled material was fractionated by SDS-PAGE with a small amount of labeled material. In this case the protein was not reduced or boiled (to avoid substantial losses of biological activity), resulting in the presence of some protein aggregates (Fig. 4B). The gel was sliced and the protein was eluted and assayed for NIF-T and CSF activities (Fig. 4C) As seen with the Ultrogel material (Fig 1), the NIF-T and CSF activities comigrated on the SDS gel and correspond to the lower molecular weight band of 22,000. This molecular weight is in good agreement with previous estimates of 25,000 for NIF-T (*1*). The relation of the higher molecular weight protein to NIF-T and CSF remains to be determined.

We have demonstrated the existence of a highly purified glycoprotein with a molecular of weight of approximately 22,000 that has both CSF and NIF-T activities. These results establish that a single human lymphokine acts on both precursor cells and mature effector cells. We recently obtained unambiguous confirmation of the identity of NIF-T and CSF at the molecular level. Independent of these studies, human granulocyte-macrophage CSF was cloned from an expression complementary DNA library prepared from Mo cell messenger RNA (*7*). The DNA sequence of this clone predicts an amino acid sequence identical to that obtained from the amino terminus of purified NIF-T/CSF (Ala-Pro-Ala-Arg-Ser-Pro-[Ser]-Pro-[Ser]). The recombinant granulocyte-macrophage CSF was expressed in COS monkey cells and the supernatant was assayed for NIF-T and CSF activities. The results demonstrate that a single human gene encodes a protein with both NIF-T and CSF activities (Table 1).

Our findings are consistent with results obtained in murine systems involving purified CSF-1, which stimulates growth of mononuclear phagocytes and

Table 1. Biological activities of recombinant granulocyte macrophage CSF (GM-CSF).

| Activity | Dilution of COS cell supernatant* | | | | |
|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| NIF-T† | Yes | Yes | Yes | Yes | No |
| GM-CSF‡ | 9, 10 | 15, 16 | 12, 12 | 2, 3 | 0, 0 |

*Supernatants from COS cells transfected with the expression vector alone had no NIF-T or GM-CSF activity. †NIF-T activity was assayed as described in the legend to Fig. 1. ‡Colonies per $2 \times 10^4$ low-density, nonadherent, human bone marrow cells; results are values for duplicate wells.

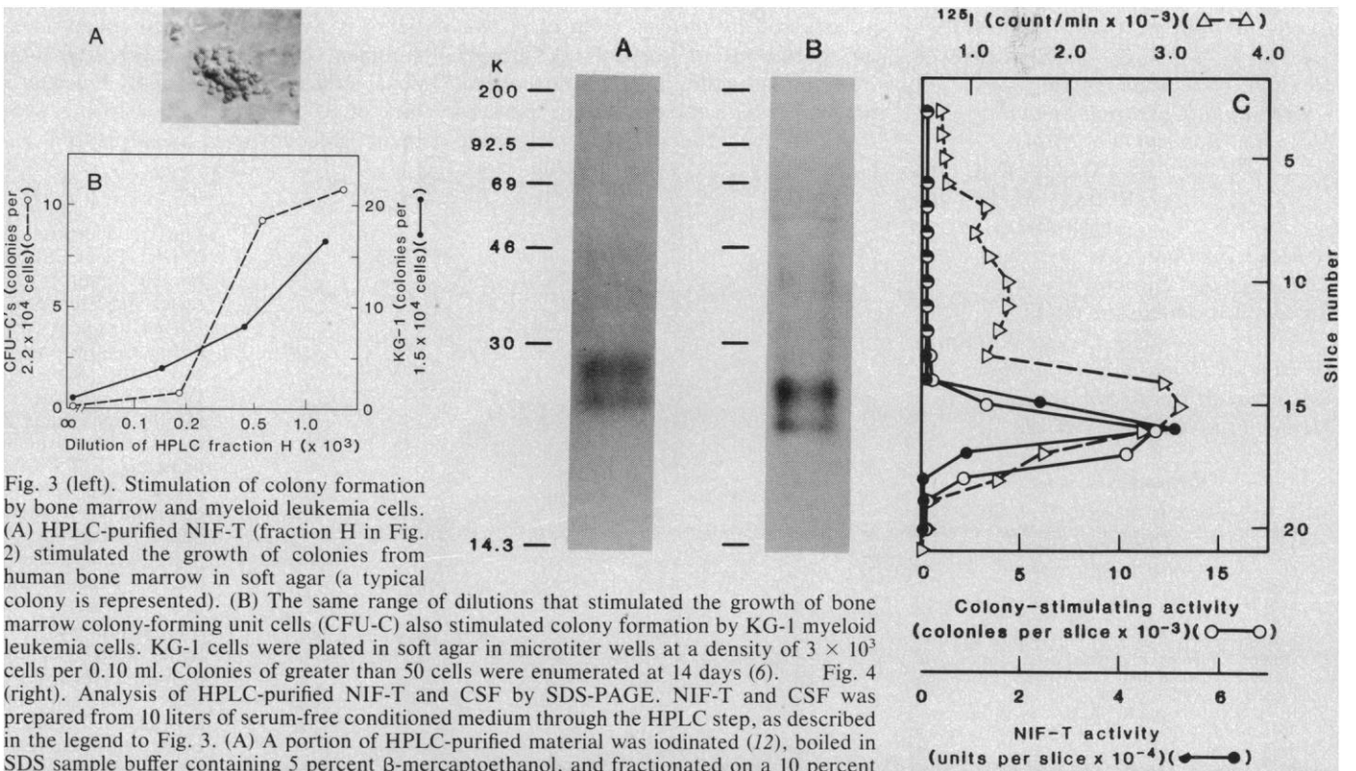


Fig. 3 (left). Stimulation of colony formation by bone marrow and myeloid leukemia cells. (A) HPLC-purified NIF-T (fraction H in Fig. 2) stimulated the growth of colonies from human bone marrow in soft agar (a typical colony is represented). (B) The same range of dilutions that stimulated the growth of bone marrow colony-forming unit cells (CFU-C) also stimulated colony formation by KG-1 myeloid leukemia cells. KG-1 cells were plated in soft agar in microtiter wells at a density of $3 \times 10^3$ cells per 0.10 ml. Colonies of greater than 50 cells were enumerated at 14 days (*6*). Fig. 4 (right). Analysis of HPLC-purified NIF-T and CSF by SDS-PAGE. NIF-T and CSF was prepared from 10 liters of serum-free conditioned medium through the HPLC step, as described in the legend to Fig. 3. (A) A portion of HPLC-purified material was iodinated (*12*), boiled in SDS sample buffer containing 5 percent β-mercaptoethanol, and fractionated on a 10 percent polyacrylamide gel (*11*). The dried gel was exposed to film. The relative positions of $^{14}C$-labeled molecular weight markers (molecular weight $\times 10^{-3}$) are given. (B) Material represented in (A) was resuspended in sample buffer containing 0.5 percent SDS without β-mercaptoethanol and electrophoresed on a 10 percent gel (*13*) along with the unlabeled protein to which iodinated protein was added as a tracer (C). The lane containing the unlabeled protein was sliced and eluted as described in the legend to Fig. 1. Colonies and clusters of greater than 25 cells from human bone marrow were enumerated at 14 days. The results of two experiments performed in duplicate were averaged. NIF-T was assayed as described in the legend to Fig. 1. Data points are means for two experiments performed in quadruplicate.

has a variety of effects on mature macrophage function and protein metabolism (*8*). In addition, purified murine granulocyte CSF and granulocyte-macrophage CSF affect murine neutrophil cytotoxic function (*9*), and semipurified human CSF from placental-conditioned medium enhances the antibody-dependent cell-mediated cytotoxicity of human neutrophils (*10*).

The physiological functions of NIF-T/CSF may be severalfold. In bone marrow this lymphokine can stimulate proliferation and differentiation of effector cells for host defense while, in the periphery, new and existing cells can be activated. In a localized immunological response NIF-T/CSF can retain circulating neutrophils in or away from areas of inflammation. Inappropriate localization or activation of neutrophils may be involved in the pathophysiology of a variety of immune-mediated disorders, such as rheumatoid arthritis. It should now be possible to study the production and function of NIF-T/CSF in normal and pathological environments.

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### References and Notes

1. R. H. Weisbart, R. Billing, D. W. Golde, *J. Lab. Clin. Med.* **93**, 622 (1979); R. H. Weisbart *et al.*, *J. Immunol.* **128**, 457 (1982); R. H. Weisbart *et al.*, *Clin. Immunol. Immunopathol.* **22**, 408 (1982).
2. I. S. Y. Chen, S. G. Quan, D. W. Golde, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7006 (1983); J. C. Gasson, I. S. Y. Chen, C. A. Westbrook, D. W. Golde, in *Normal and Neoplastic Hematopoiesis*, D. W. Golde and P. A. Marks, Eds. (Liss, New York, 1983), p. 129.
3. R. H. Weisbart and M. R. Mickey, *J. Immunol. Methods* **16**, 269 (1977); R. Weisbart *et al.*, in preparation.
4. M. J. Cline and D. W. Golde, *Nature (London)* **277**, 177 (1979); A. J. Lusis, D. H. Quon, D. W. Golde, *Blood* **57**, 13 (1981); A. J. Lusis, D. W. Golde, D. H. Quon, L. A. Lasky, *Nature (London)* **298**, 75 (1982).
5. C. A. Westbrook, J. C. Gasson, S. E. Gerber, M. E. Selsted, D. W. Golde, *J. Biol. Chem.* **259**, 9992 (1984).
6. H. P. Koeffler and D. W. Golde, *Science* **200**, 1153 (1978); A. J. Lusis and H. P. Koeffler, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5346 (1980).
7. G. Wong *et al.*, in preparation.
8. R. N. Moore *et al.*, *Lymphokines* **3**, 119 (1981); E. J. Wing *et al.*, *J. Clin. Invest.* **69**, 270 (1982); R. J. Tushinski and E. R. Stanley, *J. Cell. Physiol.* **116**, 67 (1983).
9. A. F. Lopez *et al.*, *J. Immunol.* **131**, 2983 (1983).
10. M. A. Vadas, N. A. Nicola, D. Metcalf, *ibid.* **130**, 795 (1983).
11. U. Laemmli, *Nature (London)* **227**, 680 (1970).
12. W. Hunter and F. Greenwood, *ibid.* **194**, 495 (1962).

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## Elevated Concentrations of CSF Corticotropin-Releasing Factor–Like Immunoreactivity in Depressed Patients

Abstract. *The possibility that hypersecretion of corticotropin-releasing factor (CRF) contributes to the hyperactivity of the hypothalamo-pituitary-adrenal axis observed in patients with major depression was investigated by measuring the concentration of this peptide in cerebrospinal fluid of normal healthy volunteers and in drug-free patients with DSM-III diagnoses of major depression, schizophrenia, or dementia. When compared to the controls and the other diagnostic groups, the patients with major depression showed significantly increased cerebrospinal fluid concentrations of CRF-like immunoreactivity; in 11 of the 23 depressed patients this immunoreactivity was greater than the highest value in the normal controls. These findings are concordant with the hypothesis that CRF hypersecretion is, at least in part, responsible for the hyperactivity of the hypothalamo-pituitary-adrenal axis characteristic of major depression.*

A large proportion of patients with major depression (DSM III) or endogenous depression (Research Diagnostic Criteria) exhibit hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis as assessed by measurement of plasma concentrations of cortisol (*1*), urinary free cortisol excretion (*2*), and cortisol nonsuppression in response to the synthetic glucocorticoid, dexamethasone (*3*). The site within the HPA axis responsible for this endocrine abnormality is not known. Some evidence for adrenal hyperresponsivity to adrenocorticotropic hormone (ACTH) in depressed patients is available (*4*). Moreover, measurement of plasma ACTH after oral dexamethasone has generally revealed a lack of ACTH suppression to this synthetic glucocorticoid in depressed pa-

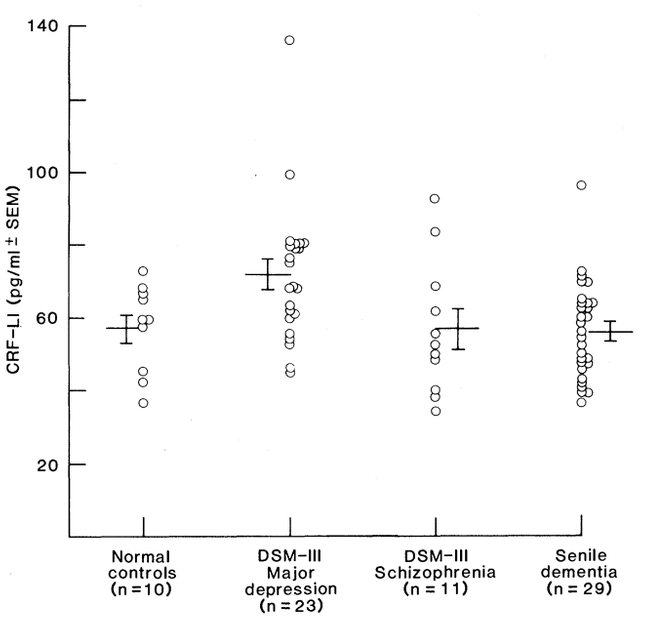


Fig. 1. Concentration of CRF-LI in CSF of normal controls (5 males and 5 females), DSM-III major depression (13 males and 10 females), DSM-III schizophrenia (8 females and 3 males), and senile dementia (DSM-III primary degenerative dementia or multi-infarct dementia, or both, 20 females and 9 males). Because the data distribution in the depressed patients was slightly skewed, the data were analyzed by both parametric (ANOVA and Student-Newman-Keuls test) and nonparametric (Mann-Whitney *U* test) methods. By both methods the CSF CRF-LI concentrations were significantly elevated in the depressed patients when compared to the other diagnostic groups and the normal controls ($P < 0.05$, ANOVA and Student-Newman-Keuls test; $P < 0.025$, Mann-Whitney *U* test).