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$$\epsilon_{Nd}(t) = \left[\frac{(^{143}Nd/^{144}Nd)^{\epsilon}_{initial}}{(^{143}Nd/^{144}Nd)_{CHUR}} - 1\right]10^4$$

calculated for some time (t) in the past where CHUR is the chondritic uniform reservoir and "initial" refers to the $^{143}Nd/^{144}Nd$ of the sample at the time of crystallization [O. J. De Paolo and G. J. Wasserburg, *Geophys. Res. Lett.* **3**, 249 (1976)]. MORBs are depleted in large ion lithophile elements and originated from mantle sources with higher Sm/Nd ratios than OIBs which are more enriched in large ion lithophiles and originated from mantle sources with lower Sm/Nd ratios.
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37. Conservative estimates for the $\delta^{18}O$ values of pillow basalts reflect modern observations of recent sea-floor material and are based on mixtures of 10% clay ($\Delta_{clay\text{-}water} \approx +25$ per mil, at temperatures typical of modern seawater) and 90% basalt (+5.7 per mil). The basalt $\delta^{18}O$ whole-rock values change by only ~1 per mil, even though the $\delta^{18}O$ value of seawater changes by 10 per mil. The $\delta^{18}O$ value of a whole-rock gabbro interacting with ~0 per mil seawater at ~350°C will reflect the modal abundance of metamorphic ^{18}O-enriched quartz ($\delta^{18}O \cong +8$ per mil) and ^{18}O-depleted epidote ($\delta^{18}O \cong 0$ per mil), which limits the range of expected values. If seawater had a $\delta^{18}O$ value of −10 per mil rather than 0 per mil, the range of expected values would be −2 to −10 per mil.
38. This work was supported by the Natural Science and Engineering Research Council of Canada. We thank D. Scott and co-workers who collected and characterized all the samples analyzed in this study. We thank O. Levner for help with the $\delta^{18}O$ analyses and H. P. Schwarcz for bringing the Purtuniq ophiolite to our attention. Comments and suggestions by D. Scott, T. Chacko, J. Farquhar, B. Gautason, J. Staveley, and two anonymous reviewers are gratefully acknowledged.

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Released Form of CNTF Receptor α Component as a Soluble Mediator of CNTF Responses

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The α component of the receptor for ciliary neurotrophic factor (CNTF) differs from other known growth factor receptors in that it is anchored to cell membranes by a glycosylphosphatidylinositol linkage. One possible function of this type of linkage is to allow for the regulated release of this receptor component. Cell lines not normally responsive to CNTF responded to treatment with a combination of CNTF and a soluble form of the CNTF α receptor component. These findings not only demonstrate that the CNTF receptor α chain is a required component of the functional CNTF receptor complex but also reveal that it can function in soluble form as part of a heterodimeric ligand. Potential physiological roles for the soluble CNTF receptor are suggested by its presence in cerebrospinal fluid and by its release from skeletal muscle in response to peripheral nerve injury.

Originally, CNTF was described as an activity that supports the survival of ciliary ganglion neurons in vitro (*1*), but it is now known to promote the survival or differentiation (or both) of a wide assortment of cells within the nervous system (*2*). The molecular cloning of a CNTF-binding protein (here denoted CNTFRα) revealed that it was similar to one of the two components of the interleukin-6 (IL-6) receptor (here denoted IL-6Rα) (*3*, *4*). This similarity suggested that, like the IL-6 receptor, which has a signal-transducing component called gp130 (*5*, *6*), the CNTF receptor might also require additional components to effect signal transduction (*4*).

The factors CNTF and IL-6 are distantly related members of a family that includes leukemia inhibitory factor (LIF) [also known as cholinergic differentiation factor (CDF) (*7*, *8*)]; all of these factors utilize multicomponent receptors that share signal-transducing subunits (*9*, *10*). In particular, all of these receptor complexes include gp130 (*10*). Both CNTF and LIF (but not IL-6) may also share an additional receptor subunit that is related to gp130 (here referred to as LIFRβ) (*10*), which was initially identified as a LIF-binding protein (*11*). It has been suggested that gp130 and LIFRβ together form a functional LIF receptor complex (*9*, *10*) and that addition of the CNTFRα chain to this complex is sufficient to convert a functional LIF receptor into a functional CNTF receptor (*10*). Whereas the broad distributions of both gp130 and LIFRβ presumably allow for the widespread actions of LIF, the limited distribution of CNTFRα (predominantly to the nervous system) may restrict CNTF to its neuronal actions (*12*).

The CNTF receptor is unlike all other

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known growth factor receptors in that it has a receptor component (the CNTFRα chain) that is anchored to cell membranes by a glycosyl-phosphatidylinositol (GPI) linkage (*4*). Like other GPI-linked proteins, CNTFRα can be released from the cell surface by phosphatidylinositol-specific phospholipase C (PI-PLC) (*4*). This raises the possibility that CNTFRα may, in some circumstances, be released from the cell surface as a soluble molecule. The protein most closely related to CNTFRα is IL-6Rα, the only known receptor component whose soluble form can promote rather than antagonize the biological effects of its ligand (*5, 6, 13*). Soluble CNTFRα (sCNTFRα) might therefore allow functional CNTF responses in cells that do not express the entire receptor complex but do express the appropriate signal-transducing receptor components.

To investigate potential functions of sCNTFRα and to determine whether it can participate directly in the activation of signal-transducing proteins, we studied the effects of CNTF and sCNTFRα on phenotypic responses and signal-transduction events in two hemopoietic cell lines that normally respond to LIF but not to CNTF and in one hemopoietic cell line that does not respond to either factor. The two LIF-responsive cell lines were M1, a murine myeloid progenitor cell line, and TF-1, a human erythroleukemia cell line. These cells display markedly different responses to LIF. M1 cells stop proliferating and differentiate into macrophage-like cells in response to LIF (*8*). In contrast, TF-1 cells display proliferative, rather than antiproliferative, responses to LIF (*14*). The murine plasmacytoma B9 requires IL-6 for survival (*15*) but does not respond to LIF or CNTF. Consistent with models of the LIF receptor complex (*9, 10*), the LIF-responsive cell lines (M1 and TF-1) express both LIFRβ and gp130, whereas B9 expresses only gp130 (*10, 16*). None of these hemopoietic cell lines express detectable amounts of CNTFRα mRNA (*10, 16*).

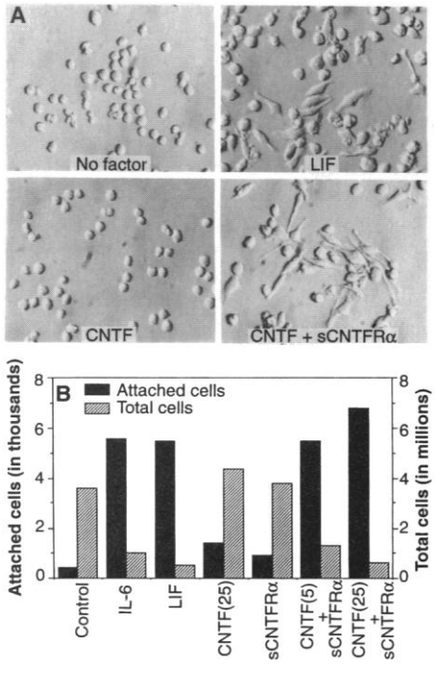

Fig. 1. Effect of sCNTFRα on proliferation and differentiation of M1 cells. (**A**) Morphological differentiation caused by CNTF and sCNTFRα. Cells were incubated for 5 days with no factor, LIF (1 nM), CNTF (5 nM), or both CNTF (5 nM) and sCNTFRα (25 nM). (**B**) Effects of IL-6, LIF, and CNTF with or without sCNTFRα on proliferation and adhesion of M1 cells. The M1 cells were incubated in 24-well plates for 5 days with IL-6 (1 nM), LIF (1 nM), CNTF (concentration indicated in figure in nM), sCNTFRα (25 nM), or both CNTF and sCNTFRα. Total cells and cells adhering to the culture dishes after three washes with phosphate-buffered saline were then counted.

Although M1 cells did not respond to CNTF (or to sCNTFRα alone) with any of the morphological changes (that is, substrate attachment, cell flattening, and elongation) that are characteristic of LIF-induced differentiation, a combination of CNTF with sCNTFRα did result in these changes (Fig. 1A). Bacterially produced sCNTFRα (thus lacking any portion of the GPI anchor) was as effective as sCNTFRα that was obtained by PI-PLC cleavage from mammalian cells (*17*). The combination of CNTF and its soluble receptor was as effective as IL-6 or LIF in inhibiting the proliferation of M1 cells and in increasing the proportion of cells that adhere to the substrate; neither CNTF nor sCNTFRα alone had any notable effect (Fig. 1B).

In contrast to M1 cells, TF-1 cells did respond to CNTF alone (Fig. 2A). However, more than 100 times higher concentrations of CNTF than of LIF were required to elicit similar proliferative responses (Fig. 2, A and B). The ability of CNTF alone to cause proliferation at very high concentrations may reflect a marginal interaction of CNTF with gp130 and LIFRβ in the absence of sCNTFRα. The addition of sCNTFRα to TF-1 cells resulted in a marked shift in the CNTF dose-response curve: CNTF became almost as potent as LIF (Fig. 2A). In contrast, sCNTFRα had only a minor effect on the LIF dose response (Fig. 2B) (*18*). The sCNTFRα had no effect on the dose response for IL-3 (Fig. 2C), which is thought not to share any receptor components with either LIF or CNTF.

The hemopoietic cell line B9, which does not respond to LIF, showed no detectable morphological or growth responses to CNTF or to the combination of CNTF and sCNTFRα. None of these factors could substitute for IL-6, which B9 cells require for survival (*16*).

The immediate early signaling events of M1 cells in response to LIF are indistinguishable from those induced by either CNTF or LIF in neuronal cells (*10*); in both cases, tyrosine phosphorylation of receptor components (gp130 and a 190-kD protein presumed to be LIFRβ) and induced transcription of a specific gene (*tis11*) have been observed. We tested whether the combination of CNTF and sCNTFRα induced these signaling events in hemopoietic cells. In M1 and TF-1 cells, the combination of CNTF and sCNTFRα (but not CNTF or sCNTFRα individually) elicited tyrosine phosphorylations (Fig. 3A) and gene inductions (Fig. 3B) that characterize CNTF responses in neuronal cells.

These results provide functional evidence that the molecularly cloned CNTFRα is directly involved in signal initiation in response to CNTF and that CNTFRα must indeed

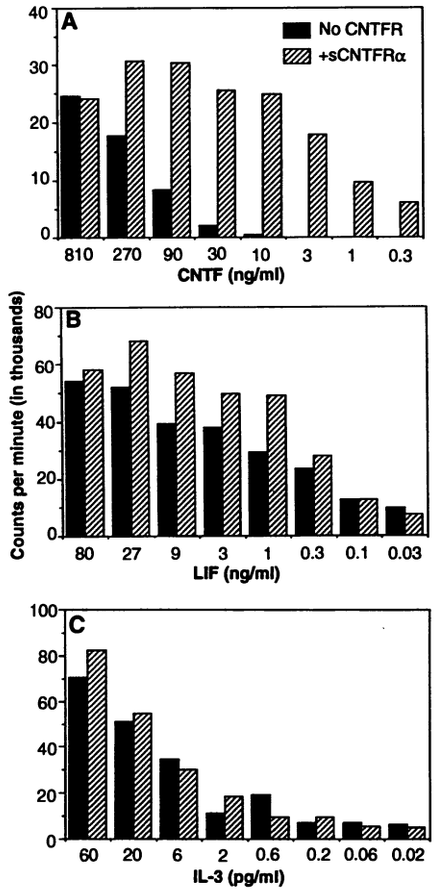

Fig. 2. Response of TF-1 cells to CNTF but not to LIF or IL-3 was enhanced by the presence of sCNTFRα. The TF-1 cells were plated in 96-well plates (1.5×10^4 cells per well) for 72 hours in the presence of serial dilutions of (**A**) CNTF, (**B**) LIF, or (**C**) IL-3, either alone (black bars) or in combination with sCNTFRα (25 nM) (hatched bars). Cells were treated for the last 4 hours with [^{3}H]thymidine, and the incorporated radioactivity was determined.

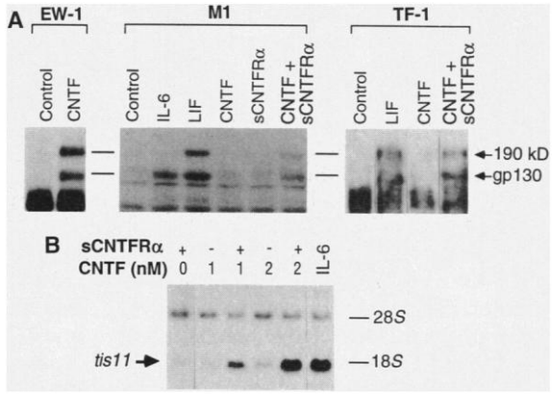

Fig. 3. Signal transduction in response to CNTF or LIF in M1 cells and TF-1 cells exposed to sCNTFRα. (**A**) Requirement of both CNTF and sCNTFRα for phosphorylation of signal transducers in M1 and TF-1 cells. Total cell lysates were prepared after treatment of cells for 5 min with IL-6 (5 nM), LIF (5 nM), CNTF (25 nM), sCNTFRα (25 nM), or both CNTF and sCNTFRα. The EW-1 cells were treated with CNTF (5 nM). Cell lysates were immunoprecipitated and immunoblotted with antibodies to phosphotyrosine. (**B**) Induction of *tis11* gene expression by CNTF and sCNTFRα. The M1 cells were treated for 45 min with sCNTFRα (25 nM), CNTF (1 or 2 nM as indicated), and IL-6 (2 nM). Total RNA was then isolated and analyzed by Northern blotting with the *tis11* gene as a probe.

interact with additional receptor components. The fact that only hemopoietic cell lines expressing both gp130 and LIFRβ respond to the combination of CNTF and sCNTFRα reveals that gp130 alone is not sufficient to transduce signals for CNTFRα and suggests that LIFRβ is a necessary component of the CNTF receptor complex.

The finding that sCNTFRα and CNTF can act on cells that are not normally responsive to CNTF raises the question of whether such effects occur in vivo. Large amounts of CNTFRα mRNA are found only within the central nervous system and in skeletal muscle (*4*); therefore, we examined both as potential sources of sCNTFRα. Substantial amounts of sCNTFRα protein (up to 10 ng/ml) were detected in several samples of human cerebrospinal fluid (Fig. 4A) (*19*), consistent with a role for the sCNTFRα in the central nervous system.

In the peripheral nervous system, CNTF may function as a Schwann cell–derived factor produced in response to nerve lesions (*20*). Schwann cells contain large amounts of CNTF and apparently release it after nerve injury. The suggestion that skeletal muscle also participates in mediating responses to peripheral nerve injury (*21*) raised the possibility that muscle-derived sCNTFRα could collaborate with Schwann cell–derived CNTF in these responses.

We examined CNTFRα mRNA expression in skeletal muscle after nerve injury. The amount of CNTFRα transcripts in rat gastrocnemius muscle increased more than 50-fold within 24 hours of nerve injury and then rapidly decreased (Fig. 4B). Our inability to detect an increase in the amount of CNTFRα protein within skeletal muscle after nerve injury was consistent with the notion that muscle might be producing CNTFRα for release. In explants derived from denervated muscle, sCNTFRα was detected in the culture medium as early as 4 hours after explantation, and then it accumulated further (Fig. 4C). In contrast, sCNTFRα was not detected until 48 hours after nondenervated rat muscle was placed in explant culture, and, even then, it was present in only small amounts (Fig. 4C).

The production of sCNTFRα by muscle during the time in which CNTF is thought to be released by injured nerve supports the proposal that these two proteins combine to evoke responses in vivo. The sCNTFRα could potentiate CNTF actions on neurons already responsive to this factor [as soluble IL-6Rα does with IL-6–responsive hemopoietic cells (*5*)]. In addition, any LIF-responsive cell, whether neuronal or nonneuronal, is a potential target for the combination of CNTF and sCNTFRα. Therefore, sCNTFRα could not only contribute to muscle-derived neurotrophic activity (*22*) but could also participate in unanticipated interactions between the nervous system and hemopoietic or other systems (*23*).

There are many examples of receptors that are found in soluble form in vivo (*24*). Almost all of these soluble receptors are thought to interfere with the binding of their ligands to membrane-bound receptors. The receptor components CNTFRα and IL-6Rα are the only ones whose soluble forms promote the actions of their ligands (*5*, *6*, *12*). Natural killer cell stimulatory factor (NKSF, or IL-12) is a heterodimeric ligand whose larger subunit, NKSF-p40, resembles both IL-6Rα and CNTFRα (*25*); NKSF-p40 is found only in soluble form. The unusual GPI-linkage of CNTFRα may participate in a release mechanism that allows CNTFRα to alternatively function either as a cell surface receptor component or as part of a heterodimeric ligand. The ability of NKSF-p40, IL-6Rα, and CNTFRα to function in soluble form may depend on a common mechanism by which these related proteins activate signal-transducing receptor components.

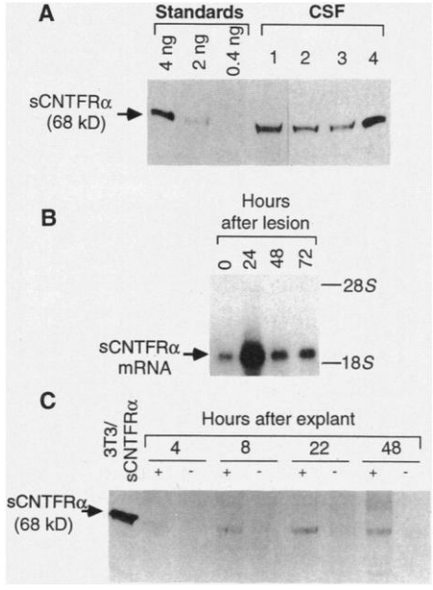

Fig. 4. Expression of sCNTFRα in the central nervous system and in response to peripheral nerve injury. (**A**) Presence of sCNTFRα in human cerebrospinal fluid (CSF). The sCNTFRα was isolated from samples of CSF (400 μl) taken from patients with lupus (lane 1), amyotrophic lateral sclerosis (lanes 2 and 4), and epilepsy (lane 3) and was detected by protein immunoblotting with an antibody to CNTFRα. Standards are known amounts of sCNTFRα isolated from supernatants of 3T3 cells treated with PI-PLC, which stably express CNTFRα. (**B**) An increased amount of CNTFRα mRNA after sciatic nerve transection increases CNTFRα message in rat skeletal muscle. The RNA was isolated from muscle samples at various times after the formation of the lesion, as indicated in the figure and was subjected to Northern analysis with ^{32}P-labeled CNTFRα cDNA as a probe. (**C**) Secretion of sCNTFRα from muscle tissue in response to denervation. Soleus muscle was dissected from normal rats (−) or rats that had been denervated 24 hours before they were killed (+). Muscle explants were incubated in 24-well plates in culture medium [0.5 ml of a mixture of Dulbecco's minimum essential medium and F12 (3:1) that contained 15 mM Hepes (pH 7.4), 3 mM histidine, 4 μM manganese chloride, and 10 μM ethanolamine]. The sCNTFRα was isolated from the culture medium (425 μl) at various times after initiation of the culture and was detected by protein immunoblotting with an antibody to CNTFRα. The standard (left lane, 3T3/sCNTFRα) was 4 ng of PI-PLC–cleaved sCNTFRα that had been isolated from culture medium.

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16. Northern analysis of RNA derived from M1, TF-1, and B9 cells was done with human or rodent probes for gp130, LIFRβ, and CNTFRα, revealing the expression patterns described in the text. In growth or survival assays done on B9 cells, substitution of LIF, CNTF, or the combination of CNTF and sCNTFRα for IL-6 resulted in cell death within 48 hours.
17. Two different methods were used to produce sCNTFRα. One method consisted of the transfection of COS cells with an expression vector that contained CNTFRα. Transfected cells were then treated with PI-PLC to release the receptor from the cells, and the supernatants were used as a source of soluble receptor. In the second method, CNTFRα without the signal sequence and the COOH-terminal GPI anchor (terminated at amino acid 349) was purified from bacteria expressing the recombinant protein (N.P., Regeneron Pharmaceuticals). Except as noted, bacterial sCNTFRα was used in all experiments.
18. The minor effects of sCNTFRα on LIF responses in TF-1 cells are perhaps consistent with a model in which CNTFRα acts as a nonessential component of the LIF receptor complex (N. Stahl *et al.*, unpublished results).
19. Protein immunoblot analysis of proteins derived from denervated muscle failed to detect an increased amount of CNTFRα compared with control samples. For the detection of the receptor in blood plasma samples, sCNTFRα was isolated by precipitation with streptavidin-agarose beads coated with biotinylated CNTF, and the resulting precipitates were subjected to protein immunoblotting with an antibody to CNTFRα. Because the size of the detected species is consistent with the PI-PLC–cleaved protein, the cleavage event that releases sCNTFRα could be in the GPI anchor. Alternatively, it could be a proteolytic cleavage that occurs close to the membrane.
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23. For example, blood-derived monocytes are thought to participate in the degeneration and regeneration of nerve axons after injury [V. H. Perry and M. C. Brown, *BioEssays* **14**, 401 (1992)]. The presence of LIF receptors on cells of the macrophage-monocyte lineage [D. J. Hilton, N. A. Nicola, D. Metcalf, *J. Cell. Physiol.* **146**, 207 (1991); *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5971 (1988)] make it conceivable that a combination of muscle-derived sCNTFRα and nerve-derived CNTF is involved in the regulation of some aspect of this process.
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26. We thank L. S. Schleifer, M. E. Furth, and the entire Discovery group at Regeneron for support; D. Everdeen for production of sCNTFRα; B. Hoffmann-Lieberman for provision of M1 cells; S. Nye for help with phosphotyrosine analysis; L. Pan and Y. Li for technical assistance; M. J. Macchi for cell culture; and M. E. Schwab for helpful discussions.

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Multiple Defects of Immune Cell Function in Mice with Disrupted Interferon-γ Genes

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Interferon-γ (IFN-γ) is a pleiotrophic cytokine with immunomodulatory effects on a variety of immune cells. Mice with a targeted disruption of the IFN-γ gene were generated. These mice developed normally and were healthy in the absence of pathogens. However, mice deficient in IFN-γ had impaired production of macrophage antimicrobial products and reduced expression of macrophage major histocompatibility complex class II antigens. IFN-γ–deficient mice were killed by a sublethal dose of the intracellular pathogen *Mycobacterium bovis*. Splenocytes exhibited uncontrolled proliferation in response to mitogen and alloantigen. After a mixed lymphocyte reaction, T cell cytolytic activity was enhanced against allogeneic target cells. Resting splenic natural killer cell activity was reduced in IFN-γ–deficient mice. Thus, IFN-γ is essential for the function of several cell types of the murine immune system.

Interferon-γ, a cytokine secreted by activated T cells and natural killer (NK) cells, has immunomodulatory effects on several cell types (*1*). IFN-γ is probably the major cytokine responsible for the activation of macrophages, which are mediators of nonspecific, cell-mediated host defense (*2, 3*). Experiments that used neutralizing antibodies to IFN-γ have shown an in vivo requirement for IFN-γ in the activation of murine macrophages for microbicidal activity and in the induction of major histocompatibility complex (MHC) class II antigen on the surface of murine macrophages (*4*). IFN-γ primes murine macrophages for production of nitric oxide (*5–7*), although other cytokines, in addition to IFN-γ, can prime macrophages in vitro for production of reactive oxygen intermediates (*7, 8*). In addition, IFN-γ may regulate the proliferation and function of activated T lymphocytes (*9–11*). Finally, NK cells exhibit enhanced cytolytic activity in response to IFN-γ in vivo and in vitro (*12*). Although IFN-γ can profoundly affect immune responses in vitro and in vivo, it is not known to what extent this cytokine is essential for the normal development and function of the immune system. To gain a better understanding of the physiological role of IFN-γ we have generated mice with a targeted mutation of the IFN-γ gene.

Mice with a nonfunctional IFN-γ gene were generated by replacing one normal IFN-γ allele in mouse embryonic stem cells with a defective allele. The targeting vector (Fig. 1) has a 2-kb neomycin-resistance gene (*neo*^{r}) inserted into exon 2, which introduces a termination codon after the first 30 amino acids of the mature IFN-γ protein. The targeting vector was transfected into AB-1 embryonic stem cells and cells were selected for resistance to G418 and 1-[2-deoxy, 2-fluoro-β-δ-arabinofuranosyl]-5-iodouracil (FIAU) (*13*). Of 960 clones screened by polymerase chain reaction (PCR) in pools of eight for a replacement event, four were identified in which a normal allele was replaced by a disrupted allele (Fig. 1D).

Injection of the 48D clone into C57BL/6J blastocysts generated chimeric mice that transmitted the mutation through the germline. Heterozygous offspring of the chimeras were intercrossed to generate mice homozygous for the targeted mutation of the IFN-γ gene (gamma knock-out or gko) (Fig. 1E). To verify inactivation of IFN-γ gene function, an IFN-γ–specific ELISA (*14*) was used to measure IFN-γ protein in

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