In Situ Hybridization Histochemistry Localization of Interleukin-3 mRNA in Mouse Brain

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The hematopoietic growth factor interleukin-3 (IL-3) promotes the proliferation and maturation of pluripotent myeloid progenitor cells. In the immune system, IL-3 is synthesized by mitogen or antigen-stimulated T lymphocytes. We demonstrate the expression of IL-3 mRNA in mouse brain by in situ hybridization histochemistry and Northern blot analysis. The IL-3 mRNA is localized in discrete areas of the brain and can be found in neuronal cell body and astrocytes. Northern analysis of cerebellar RNA, compared with mRNA extracted from WEHI-3 cells, showed a single hybridization band, approximately 1.2 kb, suggesting similar processing between brain and myeloid cells. The molecular evidence and previous observations of IL-3-like biologic activity found in the brain suggest a potential role for IL-3 in the neurobiology of the CNS.

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Materials and Methods

Reagents. All reagents unless specified were reagent grade purchased from Sigma Chemical Co, St Louis. Other reagents purchased included formamide (Fluka Chemical Corp, Hauppauge, NY), formaldehyde (Fisher Scientific, Fair Lawn, NJ), α32P-dCTP (New England Nuclear, Boston), polymeric (P & S Biochemicals, Gaithersburg, MD), salmon sperm DNA (Calbiochem, San Diego, CA), dextran sulfate (Onco, Gaithersburg, MD), nitrocellulose (Schleicher and Shuell, Keene, NH), agarose and cesium chloride (Bethesda Research Labs, Gaithersburg, MD), and 35S-dCTP (Amersham, Arlington Heights, IL).

RNA preparation and hybridization. Mouse cerebellum was isolated immediately after the brains were removed, guanidine thiocyanate was added (25 mL/g tissue), disrupted with a Brinkman homogenizer, and stored at -80°C until use. Samples were thawed and layered over a cushion of 5.7 mol/L CsCl (25 mL sample/15 mL CsCl) and centrifuged at 20,000 x g for 18 hours. The protein and DNA layers were carefully removed, and the RNA pellet resuspended in 500 μL sterile water containing 0.2% sodium dodecyl sulfate (SDS). The RNA was heated at 65°C for 15 minutes, and insoluble material (lipid) was removed by centrifugation in a microfuge. To the purified total RNA were added 50 μL of 3.0 mol/L sodium acetate, the RNA was extracted once with phenol/chloroform (1:1), once with chloroform, and precipitated with 2.5 vol of 100% ethanol. RNA samples were resuspended in sterile water, and RNA concentrations were determined by densitometer readings at 260 nm. Control RNA was isolated as described previously from WEHI-3 cells. Total RNA (20 μg) was denatured and electrophoresed on a 1% agarose formaldehyde gel according to Lehrach et al and modified by using 0.22 mol/L formaldehyde in the gel at 120 V for three to four hours. Northern transfer was performed as described by Thomas. Hyridization was performed at 42°C in

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then kept frozen at 
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slide) onto twice subbed (gelatin-chrome alum coated) microscope 
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in a series of graded alcohols, and defatted with chloro-
were developed in D19. The slides 
emulsion-coated slides 
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to ten days.
three 
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PBS) 
phate buffered saline 
brought to room temperature, fixed in 4% formaldehyde 
In situ hybridization histochemistry. NIH Swiss mice were 
decapitated and the brains rapidly removed from the skull. Brains 
decapitated
were briefly immersed in 2-methylbutane at –40°C, patted dry with 
tissue, and frozen on powdered dry ice. The fresh frozen brains were 
cut coronally at 12 μm on a cryostat, thaw mounted (two sections per 
slide) onto twice subbed (gelatin-Chrome alum coated) microscope 
slides, and dried for two minutes on a 28 to 30°C warming tray. The 
slides were then kept frozen at –20°C until use.
The in situ hybridization technique was performed as described by 
Young et al.15 Before hybridization was initiated the sections were 
brought to room temperature, fixed in 4% formaldehyde in phosphate buffered saline (PBS) for five minutes at room temperature, dehydrated in a series of graded alcohols, and defatted with chloro-
form.
After drying, 45 μL of the hybridization buffer was pipetted over 
the sections that were then covered with a parafilm coverslip and 
icubated overnight at 38°C. Hybridization buffer was composed of 
50% formamide, 4 × SSC, 10 mmol/L DTT, 500 μg/mL sheared 
single stranded DNA, 250 μg yeast tRNA, 1 × Denhardt's, 10% 
dextran sulfate, and approximately 1 × 106 DPM of the interleukin 
1 (IL-1) probe in 45 μL of hybridization buffer.
The following day the coverslips were removed and the sections 
were rinsed in four, 15-minute washes of 2 × SSC/50% formamide 
at 40°C, two one-hour washes in 1 × SSC at room temperature 
dipped briefly in water, and rapidly dried in a stream of cool air. The 
dried sections were exposed to LKB (Uppsala, Sweden) ultrafilm 
for three to six days after which they were dipped into NTB3 nuclear 
emulsion and exposed for seven to ten days. After exposure, both 
film and emulsion-coated slides were developed in D19. The slides 
were then stained with thionin, dehydrated, cleared, and covered-
slipped.
Controls were included with the in situ procedure. First, PBR322 
plasmid probe less insert was labeled in a similar manner as insert 
and was hybridized to adjacent brain sections. Second, brain tissue 
sections were pretreated with 120 ng RNase for one hour at room 
temperature before hybridization.
RESULTS AND DISCUSSION
Autoradiography emerged in the late 1970s as a powerful 
method to examine the precise localization of specific receptors 
for neurotransmitters and drugs in brain. Such procedures have allowed us to examine the distribution of receptors 
for the monokine IL-1 in the brain,14 as well as receptors for radiolabelled IL-3.17 In situ hybridization histochemistry 
allows the annealing of complementary nucleotide sequences 
to mRNA in intact fixed tissues. Such specific hybridization 

in brain has previously localized corticotropin releasing factor15 mRNA as well as protein kinase C (PK-C) mRNA 
by methods used in this report.18 Using 35S-dCTP labeled plasmid insert cDNA for mouse IL-3, we studied the localization 
of IL-3 mRNA in coronal sections of mouse brain. 
IL-3 mRNA is located in neurons and glia in wide regions 
of the mouse brain. At the level of the thalamus (Fig 1A), 
hybridization grains were especially evident in the medial 
habenula (MH) and in cells of the neuron-dense regions of 
the brain such as the granule cell layers of the olfactory bulb, 
hippocampal formation (H), and cortex (Ctx). The pyrami-
dal cell layers of the olfactory tubercle, pyriform cortex 
(PC), entorhinal and cingulate cortex (CC) also have abundant 
IL-3 mRNA positive cells. A corresponding thalamus section pre-treated with RNase did not hybridize to labelled 
insert (Fig 1B) nor did labeled plasmid reveal any specific hybridization (not shown). At midbrain (Fig 1C) the cells of the subiculum (Su) have very dense accumulations of grains. Moderate levels of grains were seen over cells in the Ctx, especially in the superficial and deep layers. Lower clustering 
of grains were seen in the striatum (S) of the thalamus 
(Fig 1A) and hypothalamus and mamillary nuclei (MN) of 
midbrain (Fig 1C). Inferior colliculus and medulla (Fig 1D) 
showed substantial hybridization in cells in the pontine 
nuclei (PN) and Ctx. Microscopic evaluation of hippocampal 
neurons showed clustering of grains associated with the neuronal cell body (Fig 2). Cells in the deep cortical layers 
showed little hybridization and the grains randomly distrib-

![Fig 1](https://www.bloodjournal.org)
Fig 2. Microscopic analysis of emulsion dipped hippocampal sections. (A) Cells in the deep cortical layers; (B) cells of the hippocampal formation.

Cells of the hippocampus showed significant grain clustering with neurons (Fig 2b). Cultured astrocytes also were positive with IL-3 cDNA probe (data not shown).

The areas of brain observed here are uniformly vascular. Therefore, hybridization to perivascular mononuclear cells would be in a diffuse pattern at all levels of brain. Two additional facts also exclude this possibility. Unstimulated lymphocytes or cells of monocytic lineage do not synthesize IL-3 mRNA and second, the microscopic analysis of hybridization grains found them to be associated with neuronal cell bodies (Fig 2b).

Total RNA was extracted from the murine cell line WEHI-3, which constitutively produces IL-3, and from freshly isolated mouse cerebellum. Northern blot analysis of IL-3 mRNA obtained from brain and the myeloleukemic cell line is shown in Fig 3. A clear 1.2 kb hybridization band is visible from RNA obtained from the WEHI-3 cell line and the same size band was found in cerebellum tissue. Exposure rates differed in the respective RNA sources since WEHI-3 produces IL-3 in large quantities, whereas cerebellum is a heterogeneous tissue. The Northern analysis confirmed the presence of IL-3 mRNA seen in brain with in situ hybridization histochemistry and suggested a message size consistent with IL-3 derived from immune or hematologic sources.

We have described the presence of IL-3 mRNA in mouse brain through in situ and Northern molecular hybridization analysis. IL-3 mRNA is present in neurons and astrocytes and not blood-borne cells of the vasculature. First, areas of brain in which discrete hybridization occurred are diffusely vascularized and the clustering of hybridization occurs primarily in cells associated with the neuroarchitecture of the region and counter stain with Nissl, a stain which delineates cell body. Second, in the immune and hematologic tissues, IL-3 is an inducible gene. In the brain, IL-3 mRNA is regulated without apparent immune stimulus. Finally, microscopic analysis of hybridization grains revealed clustering associated with neuronal cell bodies.

IL-3-like biochemical and biologic activity has been found in mouse brain astrocytes. We have confirmed that a similar transcript is seen in cerebellum tissue (Fig 3). Brain-derived IL-3 can stimulate the growth of macrophages and their representatives in brain, the micoglia cells. IL-3 mRNA is also found in neurons, although a biologic role has not been tested. The role of IL-3, albeit unexplored, in brain neurobiology, development and behavior may not be unique to IL-3 as a representative of the family of CSFs.

We have shown the presence of IL-1 receptors in the brain as well as in IL-1β mRNA. We could not find evidence for IL-1α mRNA and found expression of only IL-1β mRNA. Like IL-3, IL-1 biologic activity has been seen in the brain and has promoted astrocyte growth. These observations have been supported by the recent findings of Breder et al who found evidence for IL-1β protein in human neurons but no expression of IL-1α protein in any areas of the brain. As an authentic monoclonal antibody against murine IL-3 become available, it will be of interest to examine whether the protein is co-expressed with mRNA. Among the many biologic effects of IL-1 studied, IL-1 has also been shown to be like hematopoietin 1, a stimulator of early progenitor cells. Biologically, IL-1 has been shown to synergize with IL-3 in the proliferation of multi-potent progenitors. In the brain, IL-3 mRNA was observed in brain areas where IL-1β mRNA and IL-1 receptors have also been localized. These are unique messages since no exon homology exists between IL-1 and IL-3 and distinct mRNA sizes are also found.

The impact of IL-3 or IL-1 on the neurobiology and pathobiology of brain as well on behavior is relatively unknown. Although IL-1 has been shown to modulate fever...
and slow-wave sleep, no such activities have been reported for IL-3. The role of IL-1 in the brain, however, appears to have substantially different biologic and physiologic effects than those involved in immune and hematopoietic regulation. The finding of IL-3 in the brain would also suggest a physiologic role that may significantly differ from data and interpretations obtained from hematopoietic tissues. The physiologic regulation of IL-3 synthesis in the brain is unknown. For instance, if changes in IL-3 synthesis levels are altered during environmental or immune stress, this may indicate some level of regulation. Otherwise, constitutive synthesis may indicate some vital function of the molecule. Our initial observations may provide a basis for future studies. It is clear that substantial biochemical biologic, and molecular evidence exists that indicate that immune and hematopoietic regulatory molecules are present in neurologic tissues. These observations must now significantly broaden the potential role of these regulatory molecules in the physiology of the host and our concepts of the biologic function of these cytokine proteins.

REFERENCES

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