per milliliter. Inoculum dose was 0.2 ml per mouse (10 μg of MTP-PE).
11. Chromatographically pure egg PC and beef-brain PS were purchased from Avanti Polar Lipids, Inc., Birmingham, Ala., and stored in sealed ampules at −70°C. MLV containing Ca^{2+}- and Mg^{2+}-free PBS or MTP-PE were prepared from a mixture of PC and PS (8:2 molar ratio) as described in (*6*). MTP-PE was incorporated into the liposomes in a ratio of 8 μg per micromole of phospholipid. Preparations of liposomes in PBS were adjusted to concentrations of 25 μmol of lipid per milliliter of solution. Inoculum dose was 0.2 ml, which contained 5 μmol of phospholipids and 40 μg of MTP-PE.
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Interleukin-1 Stimulation of Astroglial Proliferation After Brain Injury

Abstract. *The interleukins, which have a regulatory role in immune function, may also mediate inflammation associated with injury to the brain. In experiments to determine the effect of these peptide hormones on glial cell proliferation in culture, interleukin-1 was a potent mitogen for astroglia but had no effect on oligodendroglia. Interleukin-2 did not alter the growth of either type of glial cell. Activity similar to that of interleukin-1 was detected in brains of adult rats 10 days after the brains had been injured. These findings suggest that interleukin-1, released by inflammatory cells, may promote the formation of scars by astroglia in the damaged mammalian brain.*

The interleukins are a family of peptide hormones that take part in the regulation of immune function (*1*). Interleukin-2 (IL-2) is released by activated T cells and has a strict T-cell tropism (*2*). Interleukin-1 (IL-1), however, is not only secreted in considerable quantities by monocytes and macrophages (*3*) but is also released by various other cells (*4–6*). IL-1 affects a wide range of target tissues and is thought to be an important mediator of the inflammatory response by acting as a growth factor for fibroblasts (*7*), by inducing the release of prostaglandin E_2 (*8*), and by stimulating the secretion of proteases (*9*). It is possible that interleukins also mediate inflammatory responses that occur after injury to the brain (*6, 10*). Because one cellular response to brain injury is proliferation of glial cells, we tested the ability of interleukins to stimulate growth of brain glia.

Glia from brains of newborn rats were grown in dissociated cell cultures in a defined medium on cover slips coated with poly-L-lysine (*11*). Indirect immunofluorescence techniques were used to identify cell-specific markers, galactocerebroside (GC) for oligodendroglia and glial acidic fibrillary protein (GFAP) for astroglia (*12*). Mean cell numbers were determined for each culture by counting ten randomly selected fields (0.314 mm^2) of specifically stained cells as viewed by fluorescence microscopy. Control cultures with added phosphate-buffered saline (PBS) were prepared in an identical fashion. A minimum of three cultures was used for each data point.

Conditioned medium containing IL-1 was prepared from acute monocytic leukemia (AMoL) cells from patients receiving therapeutic leukapheresis (*13*). The IL-1 was purified from the conditioned medium by ultrafiltration and diafiltration (*14*) and by either anion-exchange chromatography or isoelectric focusing (*15*). Activity of IL-1 was measured by the murine thymocyte assay (*16*). The activity of a preparation of recombinant human IL-2 (Sandoz) was

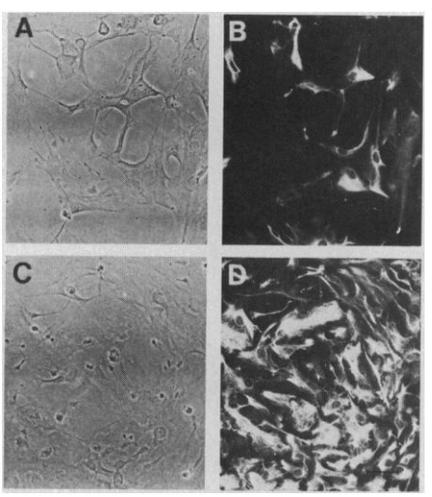

Fig. 1 (left). Photomicrographs of astroglia after treatment with partially purified human IL-1. Phase microscopy (A and C) shows the effects of IL-1 on cultures of rat brain glial cells. Cultures were treated for 3 days with either 5 percent (by volume) of the 10- to 30-kD partially purified IL-1 (C and D) or PBS (A and B). As revealed by immunofluorescence staining for glial fibrillary acidic protein, IL-1 stimulated the number of astroglia (D). Scale bar, 25 μm.

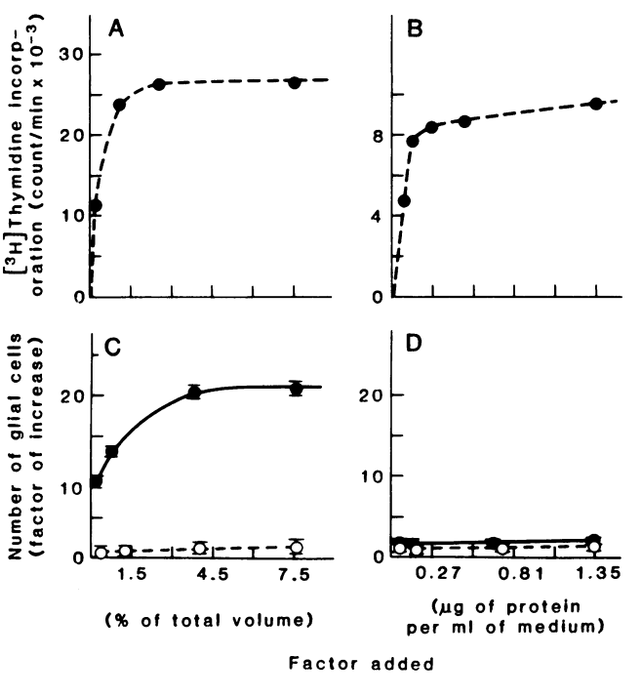

Fig. 2 (right). Dose-response curves showing the effects of partially purified IL-1 (A and C) and recombinant IL-2 (B and D) on responsive cell types. (A) The mitogenic effect of human IL-1 purified by ultrafiltration and isoelectric focusing upon murine thymocytes after 3 days of culture. The conditions for the preparation and purification of the IL-1 have been described (*14, 15*). The direct mitogenic effect of human IL-1 on murine thymocytes is well established (*16*) and is shown here as reference for the comparable effect upon astroglia (C). (B) The mitogenic effect of recombinant human IL-2 on the responsive HT-2 cell line (*2*) is shown as reference for subsequent experiments (D). (C) Concentrations of partially purified IL-1, which were shown to increase incorporation of ^3H-labeled thymidine by murine thymocytes, were also shown to increase the number of astroglia (●), but not oligodendroglia (○), in culture 3 days after the addition of IL-1. (D) Concentrations of recombinant IL-2, which increased incorporation of ^3H-labeled thymidine by the responsive HT-2 cell line, were not able to stimulate the growth of either astroglia or oligodendroglia in culture.

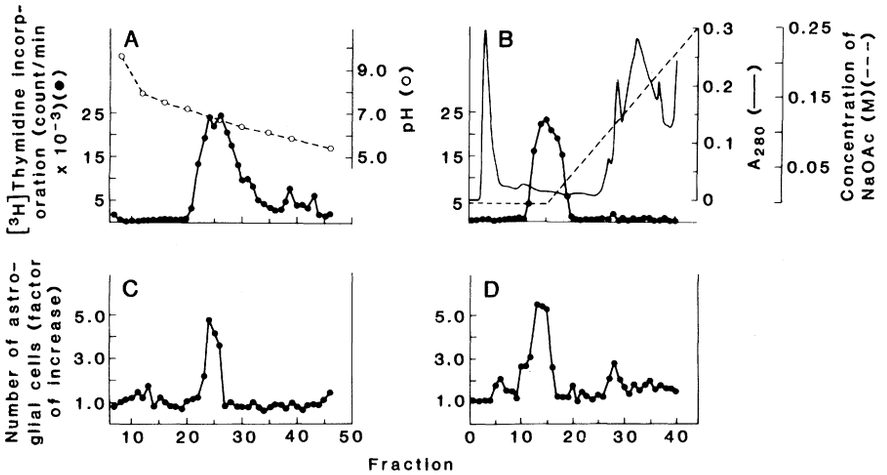

Fig. 3. Copurification of IL-1 and the astroglial stimulating activity by isoelectric focusing (A and C) and anion-exchange HPLC (B and D). A stimulating effect of small amounts of Ampholine (LKB Instruments), which may not have been completely removed by dialysis, was not considered significant because the anion-exchange chromatography also confirmed a similar isoelectric point for the two activities and was performed in the absence of Ampholine.

confirmed by means of the HT-2 cell assay (*2*). Purified IL-2 was obtained from the Jurkat cell line (*2*).

The initial study showed that a 10- to 30-kD (kilodalton) fraction containing IL-1 increased proliferation of astroglia (Fig. 1). Dose-response curves indicated that IL-1 increased the number of astroglia by a factor of about 20 compared to control preparations (Fig. 2), whereas the IL-2 from either source had no effect. Neither IL-1 nor IL-2 stimulated proliferation of oligodendroglia despite the high concentrations tested.

To confirm that the glial stimulatory factor was IL-1, we monitored the ability of all fractions of the crude IL-1 preparation to stimulate proliferation of astroglia. Only the 10- to 30-kD fractions showed this ability. This fraction from AMoL cells was purified further by anion-exchange high-performance liquid chromatography (HPLC) or by isoelectric focusing. Both the glial stimulatory factors and IL-1 showed a parallel elution profile by DEAE-HPLC (*15*) and the same isoelectric point of *p*H 7.1 (Fig. 3).

To study possible functions of IL-1 within the central nervous system, we damaged the cerebral cortex of anesthetized adult rats by delivering multiple stab wounds with a 26-gauge needle. Ten days after injury, a soluble fraction of peptides ranging from 12 to 18 kD was obtained from the damaged cortex by gel filtration through a P-10 column (100 by 0.9 cm) and by elution with PBS.

The cortex from the uninjured contralateral portion of each rat brain provided control tissue extract. A dose-response curve showed that the injured brain contained thymocyte-stimulating activity greater than that of the normal cortex by a factor of at least 3 (Fig. 4). The results of initial anion-exchange chromatography confirmed the presence of IL-1 within the extract of injured brains (*15*).

If nerve fibers in the adult mammalian brain are transected, minimum regrowth of axons and permanent loss of function occurs (*17*). Typical glial responses to such neuronal damage include the early appearance of reactive microglia and subsequent astroglial scarring (*17*). Recent studies (*18*) support the long-standing notion that microglia arise during embryogenesis from monocytes. Such cells may be one source of IL-1 in the central nervous system. Our findings suggest that IL-1 plays a role in regulating the growth response of astroglia to brain injury either directly, by acting on astroglia, or indirectly, by stimulating the release of other growth factors. It is possible that inflammatory cells at the site of brain damage release IL-1, which stimulates astroglial scarring. The source of brain IL-1 is unclear but may be astrocytes, as proposed by Fontana (*6*), invading monocytes, or microglia that appear at the site of injury. Because it has been suggested that the glial response to injury determines in part the success or failure of axonal regeneration, characterization of factors that control the growth of glial cells may help in developing treatments for damage to the central nervous system (*19*).

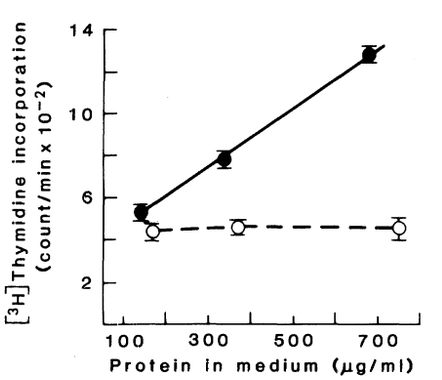

Fig. 4. Dose response of a 12- to 20-kD fraction from injured rat brain upon thymidine incorporation by thymocytes. Symbols: (●) injured tissue; (○) intact tissue.

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12. M. C. Raff *et al.*, *Nature (London)* **274**, 813 (1978). Cells adhering to the cover slips were washed three times with Dulbecco's minimum essential medium (DMEM; Gibco) containing 1 percent heat-inactivated FBS (Gibco). Identification of cells that contained GFAP was done by an indirect immunofluorescence technique. Cells were fixed at −20°C for 30 minutes with 90 percent acetone and 10 percent acetic acid (by volume). Cover slips were dipped sequentially in 100 percent acetone, 70 percent ethanol, PBS, and DMEM–1 percent FBS. Rabbit antiserum to human GFAP (Dako) was diluted 1:200 with DMEM containing 1 percent goat serum and was applied directly to the cover slips. Cover slips were incubated for 45 minutes at 37°C in a high-humidity atmosphere (95 percent air, 5 percent CO_2) and washed with DMEM–1 percent FBS. A goat antibody to rabbit immunoglobulin G conjugated with rhodamine isothiocyanate and diluted 1:100 with DMEM–1 percent goat serum was then applied for 45 minutes at 37°C. Cover slips were washed five times with DMEM–1 percent FBS and mounted in medium containing *p*-phenylenediamine (1 mg/ml), PBS (10 percent), and glycerol (90 percent) at *p*H 8.0. Identification of cells that contained GC was carried out in a similar fashion with fixation of cells after antibody binding. Rabbit antiserum to GC and a similar serum produced in our laboratory were diluted 1:50 and 1:100 with DMEM–1 percent goat serum.
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14. IL-1 was separated rapidly from the AMoL cell culture medium by hollow-fiber and membrane ultrafiltration. Culture medium (8 liters) was thawed and gravity-filtered through Whatman paper to remove debris. The sample was concentrated to 1 liter by means of a hollow-fiber device (50,000 molecular weight cutoff; Amicon), and both the ultrafiltrate and retained material were saved. The retained material was diafiltered by means of the same hollow fibers with 10 liters of 0.85 percent NaCl, and the diafiltrate and previous ultrafiltrate were pooled (17 liters). The sample was ultrafiltered with a 2-liter stirred cell (Amicon) containing a YM30 membrane (30,000 molecular weight cutoff). The ultrafiltrate (~17 liters) was retained and concentrated to 40 ml with the same stirred cell containing a YM10 membrane (10,000 molecular weight cutoff).
15. For HPLC, a 10-ml sample was dialyzed against water for 3 days and lyophilized. The sample was hydrated in 2.0 ml of 0.02*M* tris–acetic acid (*p*H 8.0, buffer A) and applied to a TSK DEAE–5-PW column (75 by 7.5 mm; Bio-Rad) at a flow rate of 1.0 ml/min. The column was developed with the indicated gradient of buffer A containing 0.5*M* sodium acetate (Fig. 3). Individual fractions were assayed by the thymocyte assay at 0.001 percent (by volume). Sucrose-gradient isoelectric focusing of a 5-ml sample of the 10- to 30-kD fraction was performed in a *p*H 4 to 8 gradient as described (*16*). Individual fractions were dialyzed for 72 hours against 0.85 percent NaCl before the thymocyte or glial cell assays.
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A Specific 37,000-Dalton Protein That Accumulates in Regenerating but Not in Nonregenerating Mammalian Nerves

Abstract. *A 37-kilodalton protein is synthesized at higher rates in the peripheral and central nervous system of newborn rats than in adult animals. As a specific response to denervation, the synthesis of the 37-kilodalton protein is increased in the mature peripheral and central nervous system; however, this protein accumulates only in the peripheral nervous system. The differences in accumulation of the protein correlate with the apparent differences in the ability of peripheral and central axons to regenerate. The synthesis of the 37-kilodalton protein is inhibited when proper innervation or reinnervation is established.*

Axons in the peripheral nervous system (PNS) of mammals can regenerate after injury, whereas axons in the adult mammalian central nervous system (CNS) typically show only abortive sprouting after lesions (*1–3*). Experiments showing extensive elongation of central axons into peripheral nerve grafts (*4*) suggested that the microenvironment of regrowing axons plays a decisive role in the success or failure of regeneration. Additional evidence supporting this hypothesis was obtained from experiments showing that PNS axons, which are known to have a capacity to regenerate, did not elongate into an environment of CNS glia (*3, 5*). Molecular interactions between axons and their adjacent glial

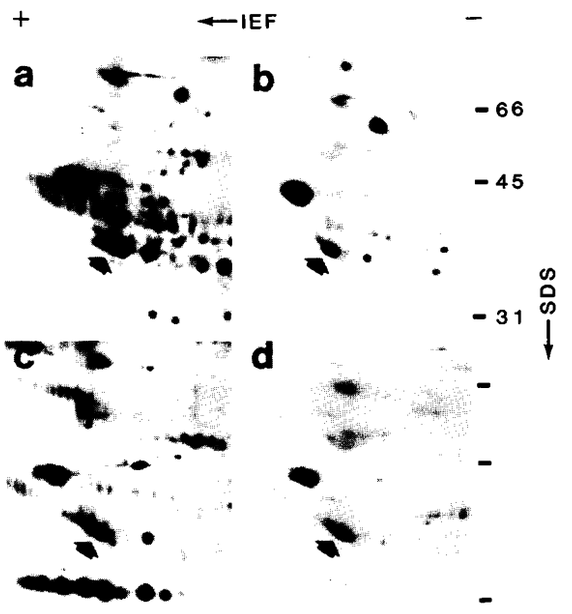

Fig. 1. Synthesis and secretion of the 37-kD protein in the developing PNS and CNS of newborn rats. Analyses of isotopically labeled proteins corresponding to (a) four sciatic nerves (wet weight of tissue, ~7 mg by weight), (b) a 0.5-cm segment of spinal cord (12 mg by weight), (c) eight optic nerves (4 mg by weight), and (d) a portion of cerebral hemisphere (20 mg by weight) are shown. The dissected nervous tissue was chopped and incubated with 100 μCi of [^{35}S]methionine in Dulbecco's modified Eagle's medium for 4 hours at 37°C. Extracellular soluble and secreted proteins released by the nerves were collected in the incubation medium, which was then dialyzed against 50 m*M* ammonium acetate before lyophilization. The dried protein samples were analyzed by 2D PAGE as described (*7*). The first dimension was isoelectric focusing (IEF) in 4.2 percent polyacrylamide gels containing 4 percent ampholytes at *p*H 3.5 to 10 and 2 percent ampholytes at *p*H 4 to 6 (LKB-Produkter AB). The second dimension was carried out in 10 percent polyacrylamide gels containing sodium dodecyl sulfate under reducing conditions (*12*). Gels were stained with Coomassie brilliant blue before autoradiographic image enhancement with EN^{3}HANCE (New England Nuclear) and then dried and exposed to presensitized Kodak XAR-5 film. The amount of protein in a, b, and c was approximately 50 μg and in d was 5 to 10 μg. The arrows indicate the position of the 37-kD protein. The numbers to the right indicate molecular mass standards (Bio-Rad): bovine serum albumin (66 kD), ovalbumin (45 kD), and carbonic anhydrase (31 kD).