Colony-Stimulating Factors as Promoters of Ameboid Microglia

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Immunomodulators were tested for their ability to stimulate proliferation and biologic activity of ameboid microglia. Only the colony-stimulating factors (CSFs), multipotential-CSF (multi-CSF) and granulocyte/macrophage-CSF (GM-CSF), were potent mitogens for microglia. Other immunomodulators, including interleukin-1, interleukin-2, interferon gamma, tumor necrosis factor, or granulocyte-CSF (G-CSF), had no effect upon microglial growth *in vitro*. Multi-CSF or GM-CSF were also observed to induce more rapid phagocytosis of polystyrene microspheres by cultured ameboid cells.

In order to determine which immunomodulators alter brain inflammatory responses *in vivo*, we infused recombinant forms of GM-CSF, multi-CSF, macrophage-CSF, or G-CSF into the cerebral cortex of rats. Within 48 hr after infusion multi-CSF or GM-CSF stimulated the appearance of large numbers of mononuclear phagocytes at the site of injection. These same factors also accelerated the clearance of polystyrene microspheres from the brain. Our observations indicate that certain classes of immunomodulators which are mitogens and activators of ameboid microglia *in vitro* amplify the inflammatory response of the CNS *in vivo* by action upon intrinsic brain mononuclear phagocytes.

The activation of mononuclear phagocytes is an important, early cellular response to brain injury (Giulian, 1987). Inflammatory cells migrate to the site of CNS damage, proliferate, release a variety of secretory factors, and engulf tissue debris (Rio-Hortega, 1932; Giulian, 1987). A growing body of evidence indicates that mononuclear phagocytes are effector cells in the brain that mediate not only the acute inflammatory response (Oehmichen, 1983) but also the subsequent astrogliotic reaction and neovascularization occurring at wound sites (Giulian and Lachman, 1985; Giulian et al., 1986a, b, 1988a). The regulatory factors to control migratory, proliferative, phagocytic, and secretory functions of brain mononuclear phagocytes are unknown.

The 2 major classes of mononuclear phagocytes in the CNS represent intrinsic and extrinsic brain cell populations (Rio-Hortega, 1932; Giulian, 1987). Blood-borne macrophage are extrinsic cells that invade the brain at sites of injury as demonstrated in penetrating wounds (Rio-Hortega, 1932; Konigsmark and Sidman, 1963; Ling, 1981; Giulian, 1987). In contrast, microglia are intrinsic brain cells that have an ameboid morphology during embryogenesis (Ling, 1981; Matsumoto and

Ikuta, 1985; Giulian and Baker, 1986; Giulian et al., 1988b) and a ramified shape in mature normal brain (Rio-Hortega, 1932; Giulian and Baker, 1986). As proposed by Rio-Hortega (1932), ramified microglia revert to the ameboid type at the time of CNS damage (Kreutzberg and Barron, 1976; Kreutzberg and Graber, 1986; Giulian, 1987). Thus, both intrinsic and extrinsic brain mononuclear phagocytes take part in CNS inflammation with the type of injury determining the composition of the inflammatory cell response (Konigsmark and Sidman, 1963; Kreutzberg and Barron, 1976; Boya et al., 1979; Brierley and Brown, 1982; Innocenti et al., 1983; Giulian, 1987). Although ameboid microglia have macrophage-like properties (Rio-Hortega, 1932; Giulian and Baker, 1986; Giulian, 1987), *in vitro* studies show that these cells may be distinguished from other classes of mononuclear phagocytes by morphology, histochemical features, and proliferative capacity (Giulian and Baker, 1986; Giulian, 1987). We sought to uncover signals that influence the biologic actions of ameboid microglia.

Colony-stimulating factors (CSFs) are glycoproteins that regulate the survival, proliferation, and differentiation of hematopoietic cells, including mononuclear phagocytes (Metcalf, 1985). Four types of CSFs have been identified (Clark and Kamens, 1987), each with varying degrees of specificity of action upon bone marrow-derived progenitor cells: granulocyte-CSF (G-CSF), granulocyte-macrophage-CSF (GM-CSF), macrophage-CSF (M-CSF), and multipotential-CSF (multi-CSF). In addition to their effects on progenitor populations, CSFs also influence more mature mononuclear phagocytes (Metcalf, 1985; Clark and Kamens, 1987).

In order to identify signals that mediate inflammation in the CNS, we have tested recombinant forms of the CSFs as well as other immunomodulators for their ability to promote proliferation and biologic activity of ameboid microglia. Our observations suggest that multi-CSF and GM-CSF amplify inflammatory responses in the central nervous system by direct action upon intrinsic brain mononuclear phagocytes.

Materials and Methods

Glial cell cultures. Mixed glial cell cultures were prepared as described earlier (Giulian and Baker, 1985) from dissociated cerebral cortex of newborn albino rat (Holtzman, Madison, WI). Cells were placed in 35 mm plastic dishes upon poly(L-lysine)-coated glass coverslips in chemically defined medium (Bottenstein and Sato, 1979) containing 10% fetal bovine serum; after 48 hr, cells were grown in chemically defined medium.

Ameboid microglia were isolated from the brains of newborn rat using the method of Giulian and Baker (1986). Cells were grown on glass coverslips [without poly(L-lysine) coating] for 24 hr in chemically defined medium plus 10% fetal bovine serum and then transferred to chemically defined medium alone. Resident peritoneal macrophages were isolated from adult albino rats (Holtzman, Madison, WI) by the

method of Daems (1980) and grown under conditions identical to those of microglia.

Glial cell identification. Ameboid microglia were identified by fluorescence microscopy using acetylated low-density lipoprotein (ac-LDL) bound to the fluorescent probe 1,1'-dioctadecyl-1,-3,3,3',3'-tetramethyl-indo-carbocyanine (DiI) (Pitas et al., 1981; Giulian and Baker, 1986). *In vitro* labeling was carried out in chemically defined medium with 5–10 μg/ml of DiI-ac-LDL for 6 hr at 37°C. The DiI-ac-LDL was obtained from Biomedical Technologies Inc. (Cambridge, MA). Cultured ameboid cells were also visualized by monitoring phagocytosis of fluorescent polystyrene microspheres (0.7 μm, Covaspheres Particles, FX Green, Duke Scientific, Palo Alto, CA) and by histochemistry for nonspecific esterase (Koski et al., 1976).

Indirect immunofluorescence techniques were used to identify astroglia containing glial fibrillary acidic protein (GFAP) and oligodendroglia containing galactocerebroside (GC) (Giulian et al., 1986a).

Bioassays. In order to test for mitogenic effects, cell cultures grown in 1.5 ml chemically defined medium were incubated with increasing concentrations of immunomodulators and growth factors for 72 hr. Proliferation of ameboid microglia, astroglia, oligodendroglia, and peritoneal macrophage were monitored by scoring the number of specifically labeled cells in 9 randomly selected fields (0.314 mm²) from each of 3 or more coverslips. The data were expressed as fold increased over control cultures incubated with matching aliquots of PBS ranging from 1 to 50 μl/ml.

Freshly isolated ameboid microglia were plated in 96 well plates (50,000 cells/well) for 24 hr in 200 μl of chemically defined medium containing 10% fetal bovine serum. Cells were then incubated in 200 μl of chemically defined medium that contained 5 units of a CSF for 48 hr. ³H-thymidine, 3 mCi, was added to each well for the last 10 hr of the incubation period. At the end of the 48 hr incubation period, cells were frozen for 1 hr at −70°C. Radiolabeled DNA released from cells lyzed with deionized water onto glass filter paper (Skatron Harvester; Sterling, VA) was measured by liquid scintillation counting.

Phagocytic activity was measured *in vitro* with fluorescence microscopy by scoring the number of fluorescently labeled polystyrene microspheres (0.7 μm in diameter) engulfed within 12 hr at 37°C by DiI-ac-LDL(+) cells. In order to test the ability of brain to remove debris, we injected 2 μl of immunomodulators in PBS containing polystyrene microspheres (0.0005% wt/vol). Particle clearance from the brain was measured by determining the number of microspheres found in serial sections taken from the site of injection. Brains were fixed in 3.5% formaldehyde buffered with PBS, frozen in OCT compound (Tissue-Tek, Naperville, IL), and cut on a freezing microtome (Minitome, Needham, MA) into 10-μm-thick serial sections in the coronal plane.

Injection of immunomodulators into the brain. To study the effects of CSFs *in vivo*, we injected small volumes of material into the cerebral cortex of albino rats. Adult rats (250–300 gm, Holtzman, Madison, WI) were deeply anesthetized by intraperitoneal injection (0.8–1.2 ml of a mixture containing 8.5 mg/ml xylazine, 42 mg/ml ketamine hydrochloride, and 1.4 mg/ml acepromazine maleate) and placed in a stereotaxic device (David Kopf, Inc.). After the scalp was reflected, burr holes through the skull were positioned over the cerebral cortex 4.5 mm caudal to bregma and 4.0 mm lateral to the sagittal suture. Using a 5.0 μl syringe (Hamilton, Reno, NE) mounted in the stereotaxic device, we infused 1.0 μl of PBS containing an immunomodulator at a depth of 1.5 mm from the surface of the brain over a 2 min interval. Control injections of PBS alone were made in an identical location on the contralateral side. The syringe needle was slowly withdrawn and the scalp closed with wound clips. Forty-eight hours later, the animals were deeply anesthetized, and a cylindrical biopsy (2.0 × 1.0 mm) was removed rapidly from the injection site following cardiac perfusion with heparin–PBS solution (500 USP units/liter). The tissue sample was then incubated for 6 hr at 37°C in chemically defined medium containing 10 μg/ml DiI-ac-LDL. The tissue was fixed with 3% formaldehyde in PBS for 1 hr and viewed using fluorescence microscopy and photographed immediately.

Immunomodulators and growth factors. Recombinant forms of immunomodulators used in this study included human granulocyte-colony stimulating factor (rh-G-CSF) from Amgen Biologicals, murine granulocyte-macrophage colony-stimulating factor (rm-GM-CSF) from Genzyme Corporation, and human macrophage-colony-stimulating factor (rh-M-CSF), a gift from Dr. Peter Ralph, Cetus Corporation. Two forms of murine multipotential-colony-stimulating factor (multi-CSF, also referred to as interleukin 3 or IL-3) were used, a murine cell line-derived type (m-IL-3) obtained from Genzyme and a recombinant mu-
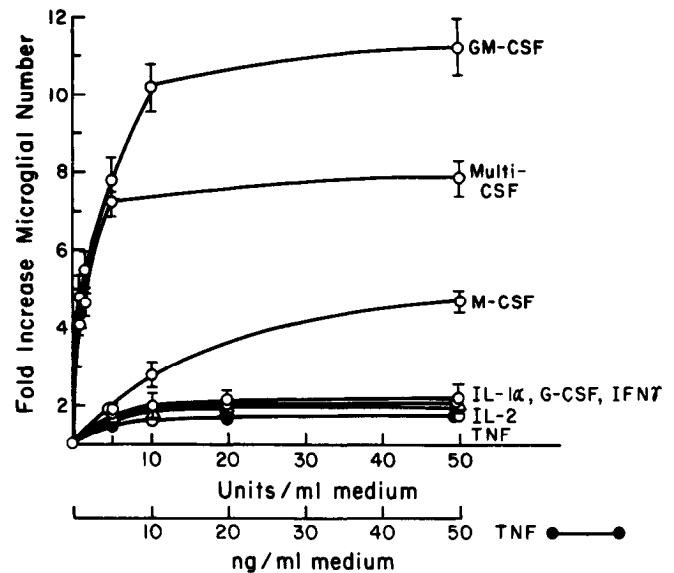
Figure 1. Proliferative effects of immunomodulators upon ameboid microglia. Mixed cell cultures from newborn rat brain were grown on coverslips in chemically defined medium containing various immunomodulators. As shown, only the colony-stimulating factors rm-GM-CSF, m-multi-CSF, and rh-M-CSF stimulated growth of microglia. Data are mean values expressed as fold increase in cell number ± SEM compared with control cultures treated with equivalent aliquots of PBS (5–50μl) that lacked immunomodulators. At least 5 cultures were used to determine each mean value.

rine form (rm-multi-CSF), which was a gift from DNAX Corp. The rm-multi-CSF (provided as a COS cell line supernatant) required further purification by high-performance liquid chromatography using a C8 reverse-phase column (4.6 × 75 mm; Beckman, Palo Alto, CA) with a 0–55% gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. Recombinant forms of human tumor necrosis factor alpha (rh-TNF alpha), human interleukin 1 alpha (rh-IL-1 alpha), human interleukin 2 (rh-IL-2), and murine interferon gamma (rm-IFN gamma) were obtained from Amgen Biologicals, Cistron Technologies, Cetus Corp., and Genentech, respectively. All purified recombinant immunomodulators were found to have less than 0.05 ng/ml of endotoxin as shown by the Limulus amebocyte lysate assay (Cape Cod Associates).
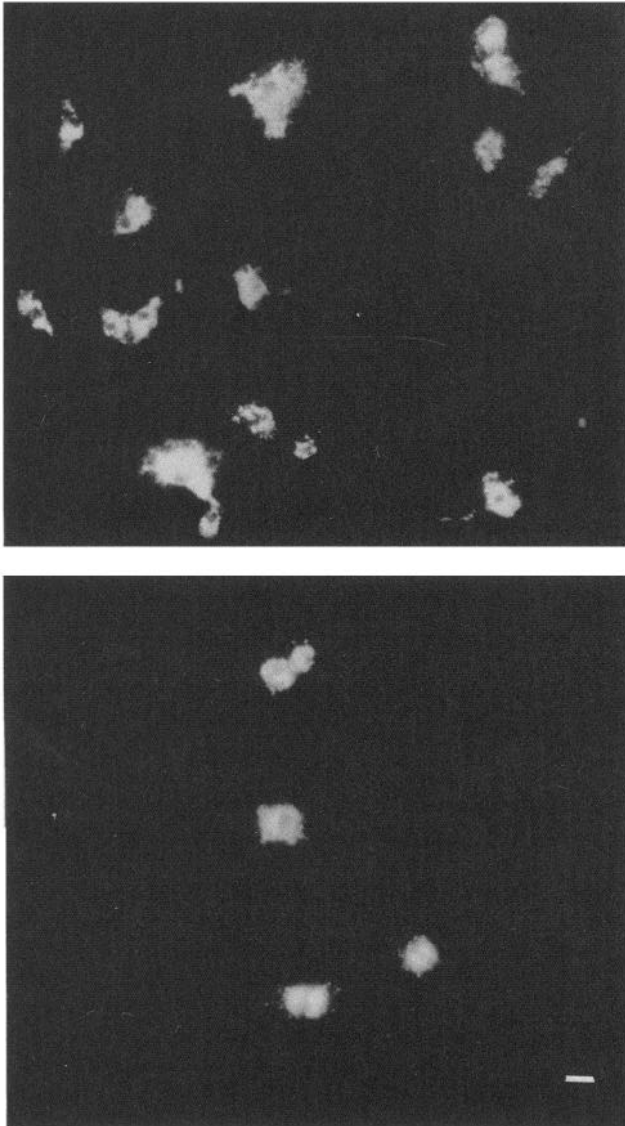
Other growth factors that were tested included recombinant human insulin-like growth factor 1 (rh-IGF-1, also referred to as somatomedin C) from Amgen Biologicals, recombinant human transforming growth factor beta (rh-TGF beta) from Amgen Biologicals, epidermal growth factor (EGF) from Collaborative Research, acidic fibroblast growth factor (FGF) from California Biotechnology, and recombinant basic FGF from Amgen Biologicals.

Results

A proliferation of ameboid microglia occurs during brain development and at sites of brain injury (Rio-Hortega, 1932; Ling, 1981). Since earlier work from our laboratory suggested that soluble factors help to regulate the number of microgroglia found in culture (Giulian and Baker, 1986), we screened a variety of immunomodulators for mitogenic effects upon ameboid cells.
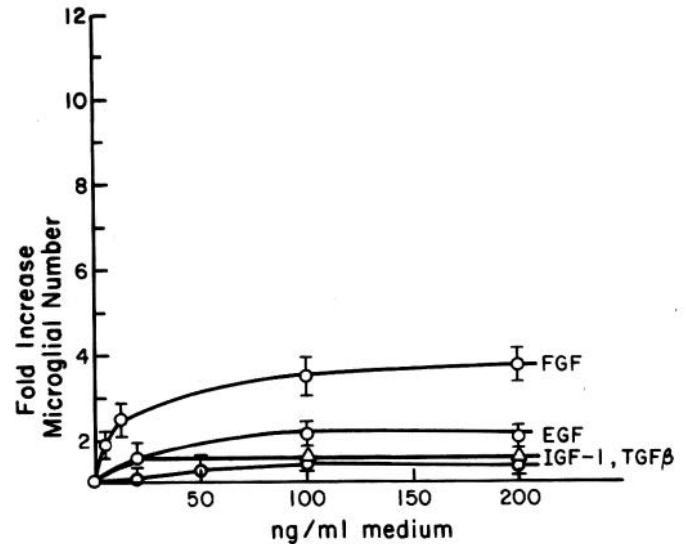
Identification of microglial mitogens

GM-CSF and multi-CSF after a 72 hr incubation period increased the number ameboid microglia found in mixed glial cultures 7- to 10-fold above that in control cultures (Fig. 1). Saturation of the mitogenic responses occurred at concentrations of about 10 units/ml for either factor. Although less effective, M-CSF (50 units/ml) also stimulated microglial growth with a 4-fold increase in cell number. Importantly, none of the

Figure 3. Proliferative effects of growth factors upon ameboid microglia. Mixed cell cultures from newborn rat brain were grown in chemically defined medium containing various protein growth factors. Only basic recombinant FGF was found to increase the number of DiI-ac-LDL(+) ameboid cells. Data are mean values ± SEM expressed as fold increase in cell number compared with control cultures. At least 5 cultures were used to determine each mean value.

Figure 2. Fluorescent photomicrographs showing the mitogenic effects of multi-CSF on mixed glial cell cultures. Cells grown in chemically defined medium containing 5 units of recombinant multi-CSF for 72 hr (*top*) had a marked increase in the number of DiI-ac-LDL(+) ameboid microglia compared with control cultures (*bottom*). Scale bar, 20 μm.

other immunomodulators examined—G-CSF, IL-1, IL-2, TNF, or IFN gamma—showed growth-promoting effects upon ameboid microglia. In testing other growth factors (EGF, FGF, IFG-1, and TGF beta), we found that only acidic and basic FGF had a small, though significant proliferative effect upon DiI-ac-LDL(+) microglia found in mixed glial cultures (Fig. 3).

Further study was necessary to determine if the mitogenic effects of the CSFs and FGF in mixed cell preparations were mediated by direct action upon microglia. Isolated ameboid microglia [>95% homogeneous DiI-ac-LDL(+) cell population] showed stimulated proliferation when incubated with GM-CSF or M-CSF (Figs. 4, 5) as found with mixed glial cultures. We also observed direct mitogenic effects of native and recombinant forms of multi-CSF (Table 1) upon ameboid microglia, as reported previously by our laboratory (Giulian and Baker, 1986) and recently confirmed by Frei and Fontanna (1986). The ac-

tions of the CSFs were cell specific, with no apparent growth-promoting effects upon GFAP(+) astroglia or GC(+) oligodendroglia (Fig. 6).

Recombinant basic FGF was not a mitogen for isolated ameboid microglia (Fig. 4), although it stimulated microglial proliferation in cultures containing astroglia and oligodendroglia (Fig. 3) In contrast, the mitogenic effects of FGF upon astroglia (Fig. 6) occurred by a direct action (Pruss et al., 1981; Giulian et al., 1986a), as shown in highly purified cell preparations. Since ameboid microglia grow rapidly atop monolayers of astroglia,
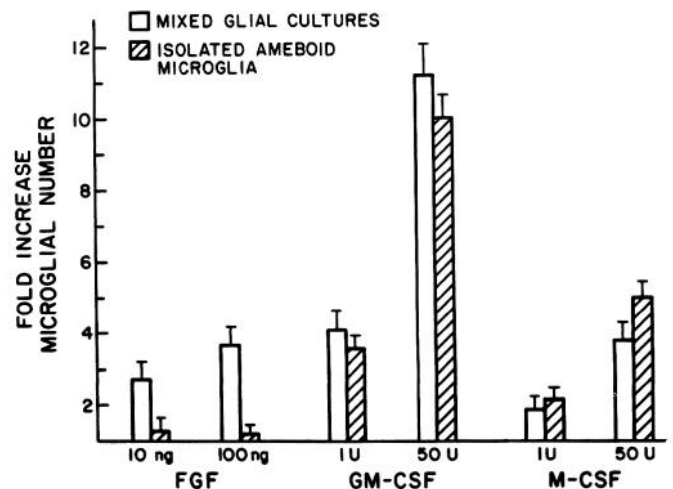
Figure 4. Direct proliferative effects of factors upon isolated ameboid microglia. Low and high doses (in 1 ml of culture medium) of recombinant basic FGF, rm-GM-CSF, and rh-M-CSF were tested for their mitogenic effects upon ameboid microglia isolated from newborn rat brain. GM-CSF and M-CSF stimulated proliferation of isolated microglia [>93% DiI-ac-LDL(+) cells]. In contrast, recombinant basic FGF only promoted growth of microglia found in mixed cultures [<10% DiI-ac-LDL(+) cells].
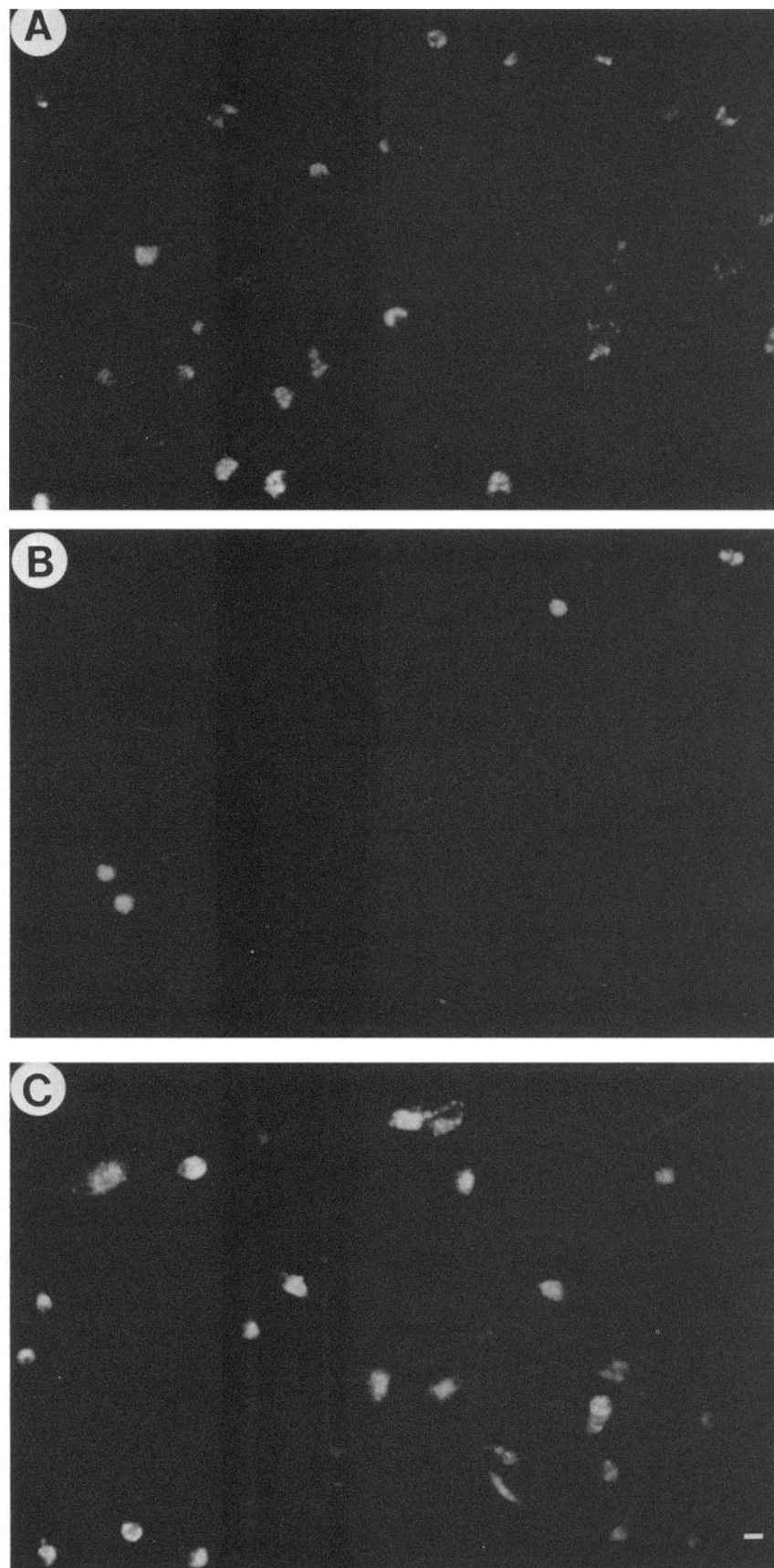
Figure 5. Fluorescence photomicrographs showing the effects of CSFs upon isolated ameboid microglia. There is a 5- to 6-fold increase in DiI-ac-LDL(+) microglia incubated for 72 hr with 5 units of rm-GM-CSF (*A*) or 50 units of rh-M-CSF (*C*) compared with those treated with 100 ng recombinant basic FGF (*B*). Scale bar, 20 μm.
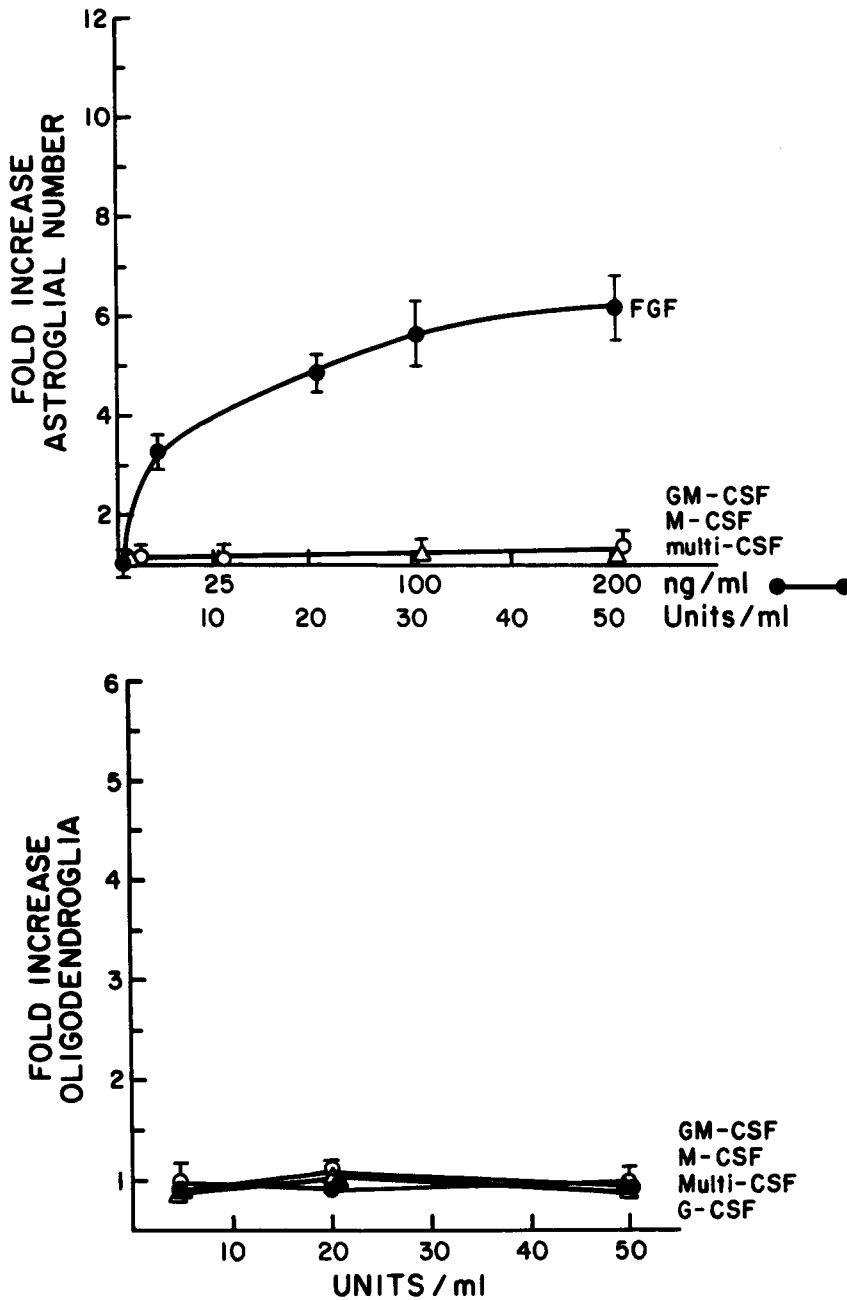
Figure 6. Growth factors and specificity of action *in vitro*. Colony-stimulating factors had no growth promoting-effects upon GFAP(+) astroglia (*top*) or GC(+) oligodendroglia (*bottom*). Recombinant basic FGF was a potent mitogen for astroglia (*top*). Data are mean values ± SEM expressed as fold increase compared with control cultures. At least 5 cultures were used to determine each mean value.

Table 1. CSFs increase ^{3}H-thymidine incorporated by ameboid microglia

Factor treatment	Incorporated ^{3}H-thymidine (counts/min)
Control ($n = 9$)	507 ± 26
Multi-CSF (3)	1623 ± 423[a]
GM-CSF (5)	1695 ± 153[b]

Freshly isolated ameboid microglia were plated in 96 well plates (50,000 cells per well) for 24 hr in 200 μl of chemically defined medium containing 10% fetal bovine serum. Cells were then incubated in 200 μl of chemically defined medium that contained 5 units of a CSF for 48 hr. Three mCi of ^{3}H-thymidine were added to each well for the last 10 hr of the incubation period. Data are expressed as mean counts per min ± SEM.

[a] $p < 0.05$.
[b] $p < 0.001$ by Student's t test using the Bonferroni correction (Godfrey, 1985).

Table 2. Effects of CSFs upon peritoneal macrophage

Factor treatment	Increase in macrophage number (fold)
Multi-CSF	1.0 ± 0.1
G-CSF	1.3 ± 0.2
M-CSF	2.5 ± 0.1
GM-CSF	1.9 ± 0.2
Basic FGF	1.1 ± 0.2

Resident peritoneal macrophage were grown on glass coverslips in chemically defined medium containing 10 units of a recombinant CSF or 100 ng FGF. After 72 hr, cells were labeled with DiI-ac-LDL. The data are expressed as mean fold increase in DiI-ac-LDL(+) macrophage ± SEM compared with untreated control cultures. Each value represents a mean score obtained from at least 5 cultures.
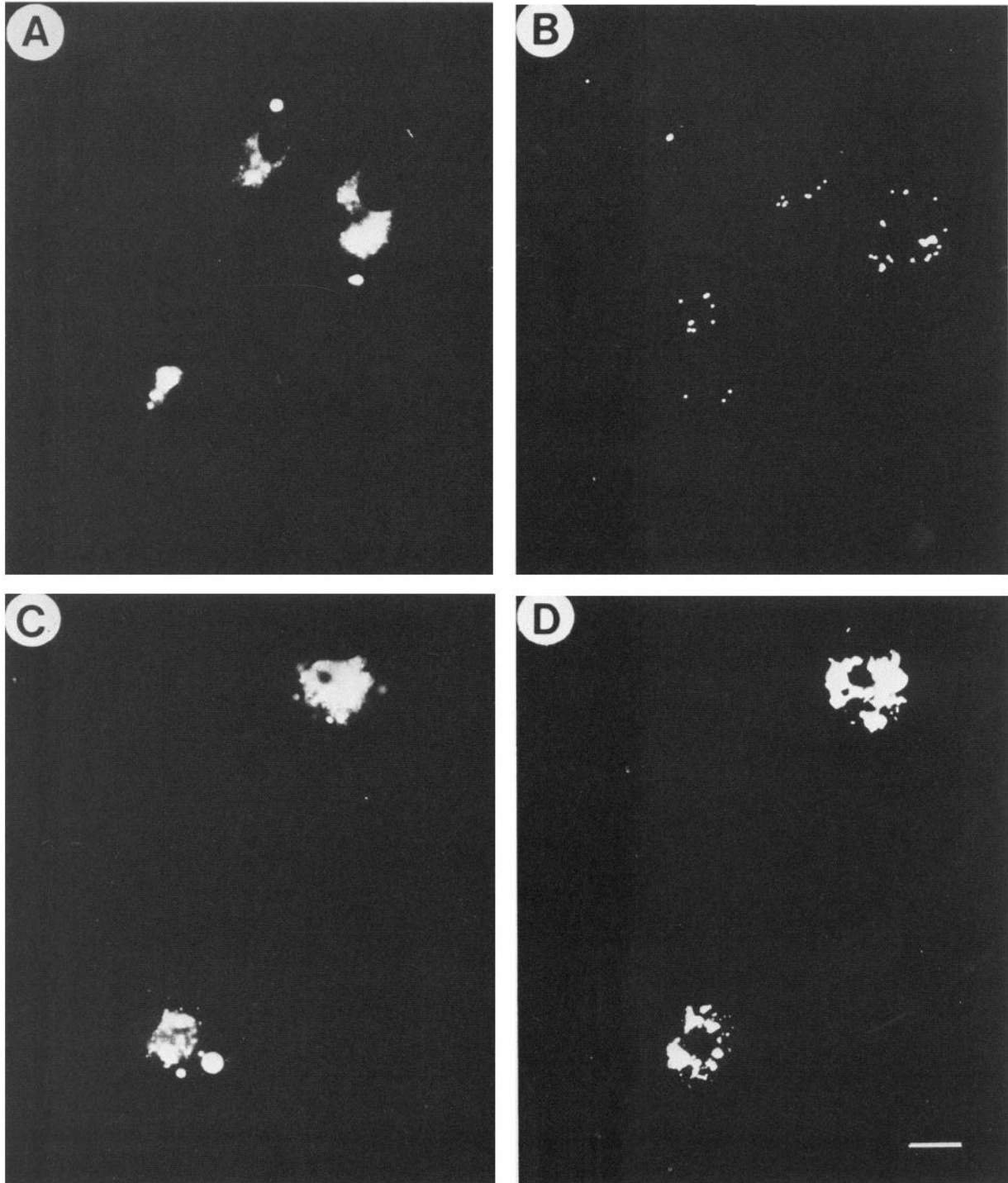
Figure 7. Fluorescence photomicrographs showing the stimulated phagocytic activity in ameboid microglia after incubation with 10 units/ml medium of rm-GM-CSF. Ameboid cells are identified by DiI-ac-LDL in control (*A*) and GM-CSF-treated (*C*) cultures. As shown in *D*, there is an increased number of fluorescently labeled microspheres engulfed by GM-CSF activated cells compared with control cells (*B*). Scale bar, 20 µm.

it is possible that FGF stimulates astroglia, which, in turn, supports rapid proliferation of microglia.

Dose–response curves (Fig. 1) indicated that GM-CSF and multi-CSF were the most potent mitogens for ameboid microglia. By contrast, M-CSF was a mitogen for resident peritoneal macrophage (Table 2), while multi-CSF had no mitogenic effect upon peritoneal cells. Such differences in the sensitivities to the action of the CSFs further support the idea that ameboid

microglia are a class of mononuclear phagocyte that may be distinguished from tissue macrophage and blood monocytes (Giulian and Baker, 1986).

CSFs as activators of microglia

As reported for macrophage (North, 1978; Nathan et al., 1980; Dinarello, 1984), ameboid microglia often require activation in order to carry out immunoeffector functions (Giulian and Baker,
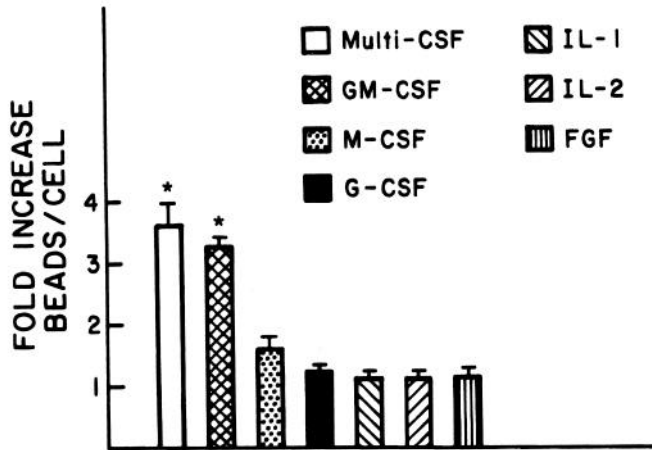
Figure 8. Activation of phagocytosis by colony-stimulating factors. Ameboid microglia in chemically defined medium containing 10% fetal bovine serum were treated with various immunomodulators or recombinant basic FGF for 6 hr at 37°C and then incubated with fluorescently labeled microspheres for 12 hr. At the end of the incubation period, cells were washed with chemically defined medium and labeled with DiI-ac-LDL. Data are mean values ± SEM expressed as the number of microspheres per cell. Concentrations (per ml) of medium included 10 units of m-multi-CSF, 10 units of rm-GM-CSF, 50 units of rh-M-CSF, 50 units of rh-G-CSF, 100 units of rh-IL-1 alpha, 100 units of rh-IL-2, and 100 ng recombinant basic FGF. *$p < 0.01$ by Student's t test is statistically significant using the Bonferroni correction (Godfrey, 1985).

1986; Giulian et al., 1986b; Giulian, 1987). We examined the phagocytic activity of ameboid microglia after incubating cells with immunomodulators prior to the presentation of fluorescently labeled polystyrene microspheres. As shown in Figure 7, more microspheres are engulfed by DiI-ac-LDL(+) cells preincubated with GM-CSF than by untreated control cells. The mean number of microspheres engulfed by ameboid cells is significantly increased after exposure to GM-CSF and multi-CSF but not to M-CSF, G-CSF, IL-1, IL-2, or FGF (Fig. 8).

CSFs amplify inflammatory responses in the adult CNS

We considered the possibility that CSFs might promote inflammation within the brain. Activated ameboid microglia or invading macrophage that appear at sites of brain injury can be identified using either the histochemical stain for nonspecific esterase (Ling, 1981) or a fluorescent probe for acetylated LDL receptors (Giulian, 1987). Normal cerebral cortex rarely contained cells that were DiI-ac-LDL(+). However, 48 hr after intracerebral injections of GM-CSF, there was a marked increase in the number of DiI-ac-LDL(+) cells at the site of injection compared with injections of GM-CSF or control injections of PBS (Fig. 9). Similar increases in the number of nonspecific esterase(+) mononuclear phagocytes were also observed at injection sites of multi-CSF (Fig. 10). Quantitation of DiI-ac-LDL(+) cells showed that GM-CSF and multi-CSF were the factors most effective in stimulating the appearance of mononuclear phagocytes within the brain (Fig. 11).

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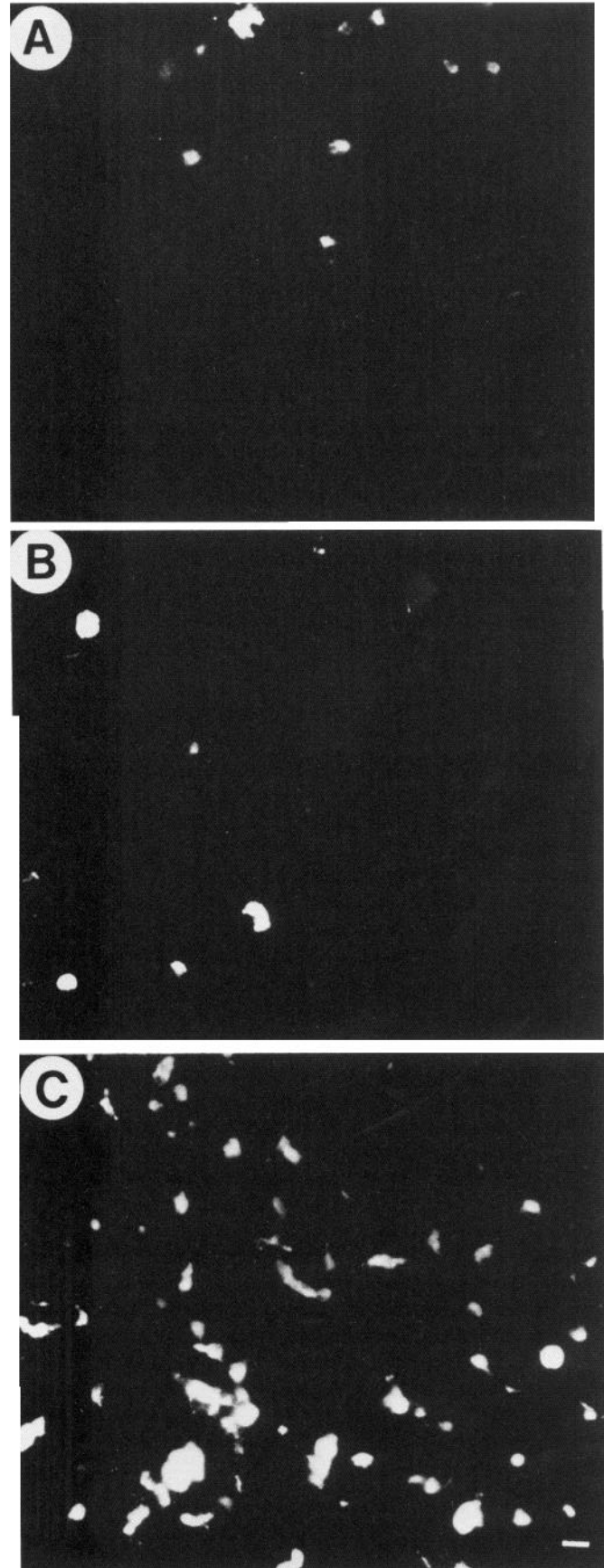
Figure 9. Fluorescence photomicrographs show the increased number of DiI-ac-LDL(+) cells within 48 hr after injection of 10 units of rm-GM-CSF (*C*) into the cerebral cortex of adult rats compared with control

PBS injection sites (*A*) or to sites with 50 units of rh-M-CSF (*B*). Scale, 20 μm.
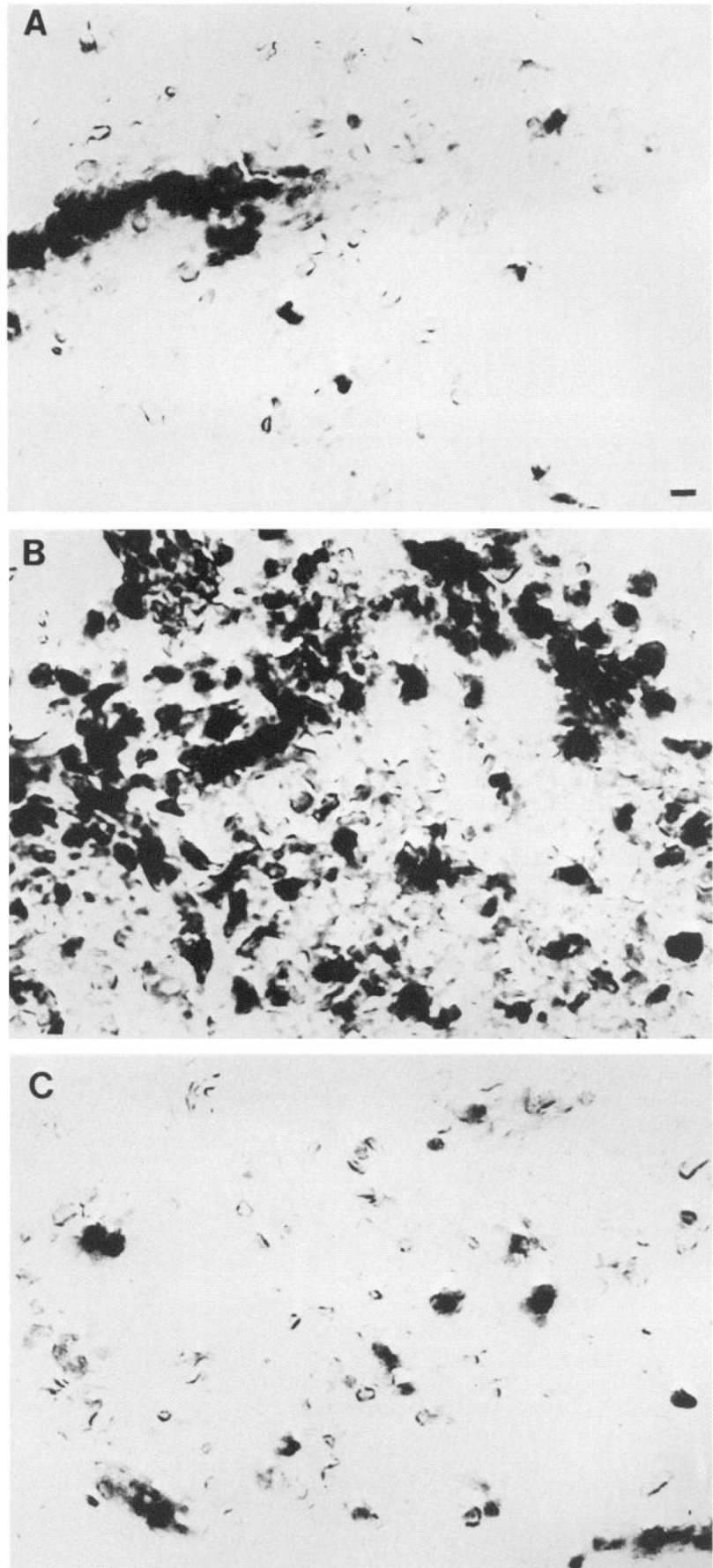
Figure 10. In vivo effects of colony-stimulating factors upon adult brain. At 48 hr after injection of 10 units of m-multi-CSF (*B*), there was a significant increase in the number of nonspecific esterase(+) cells compared with those sites receiving 50 units of rh-G-CSF (*A*) or PBS alone (*C*). Scale bar, 20 μm.
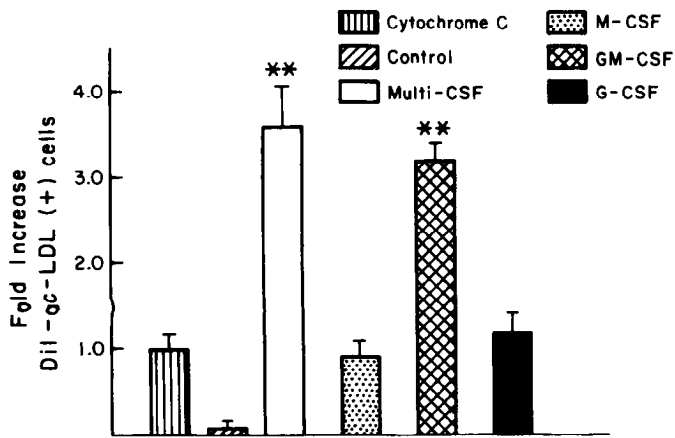
Figure 11. In vivo effects of colony-stimulating factors upon adult brain. At 48 hr after infusion of 10 units of m-multi-CSF or rm-GM-CSF, there was a significant increase in the number of DiI-ac-LDL(+) cells found at the site of injection compared with an infusion of 100 ng cytochrome C. Infusions of 50 units of rh-M-CSF or rh-G-CSF did not alter cell number. Data are mean values ± SEM expressed as the fold increase in total number of DiI-ac-LDL(+) cells seen per injection site compared with PBS vehicle injection. Uninjected sites of cerebral cortex (control) were free of DiI-ac-LDL(+) cells. Each mean value represents at least 5 injections. ** $p < 0.005$ by Student's t test using the Bonferroni correction (Godfrey, 1985).

Clearance of debris is an important function of brain mono-nuclear phagocytes (Rio-Hortega, 1932) and involves a series of cellular behaviors, including chemotaxis to the site of injury, debris recognition, phagocytosis, and movement of debris-laden cells. Fluorescently labeled polystyrene microspheres were co-injected with GM-CSF, M-CSF, G-CSF, or multi-CSF into the cerebral cortex of normal adult rats. We found a more rapid clearance of microspheres at those sites stimulated with GM-CSF or multi-CSF (Fig. 12, Table 3). Thus, specific immuno-modulators not only stimulated the proliferation and phagocytic activity of ameboid microglia in vitro but also stimulated the appearance and activity of brain mononuclear phagocytes in vivo.

Discussion

Recent work has shown that ameboid microglia are important effector cells of the CNS that act as phagocytic scavengers, se-cretors of cytotoxins, and producers of growth factors (Giulian, 1987). In this study we investigated the ability of immunomodu-lators to influence biologic activity of microglia. We found that only GM-CSF and multi-CSF were potent mitogens for ame-boid, as well as activators of phagocytosis. When injected di-rectly into the cerebral cortex of rats, GM-CSF and multi-CSF, but not M-CSF or G-CSF, increased the number of mononuclear phagocytes found at the site of infusion. GM-CSF and multi-CSF also accelerated the clearance of foreign particles from the CNS. We conclude that GM-CSF and multi-CSF are potent activators of brain mononuclear phagocytes and may serve as mediators of inflammatory responses in the brain. Although cell markers to distinguish invading macrophage from ameboid mi-croglia are not yet available, our in in vitro data (Table 1) favor the notion that GM-CSF and multi-CSF directly stimulate mi-croglia in vivo.

Multi-CSF is a mitogen for progenitor hematopoietic cells (Clark and Kamens, 1987). We found multi-CSF to be a mitogen for ameboid microglia but not newborn rat blood monocytes

Table 3. CSFs accelerate clearance of microspheres from the cerebral cortex

Factor	Microspheres per section	Clearance score (%)
Control ($n = 144$)	201 ± 25	0
M-CSF (168)	170 ± 24	15
GM-CSF (144)	39 ± 19[a]	80
G-CSF (48)	234 ± 84	−15
Multi-CSF (77)	43 ± 25[a]	80

Polystyrene microspheres (0.7 μm in diameter) were coinjected with CSFs at a depth of 1.5 mm into the cerebral cortex of adult rats. The total volume of the injections was 1 μl of suspension of microspheres in PBS containing 50 units of rh-M-CSF, 10 units of rm-GM-CSF, 50 units of rh-G-CSF, or 10 units of m-multi-CSF. The injection control was 1 μl of the microsphere-PBS suspension. Animals were sacrificed after 72 hr and the brains cut in the coronal plane with serial sections of 10 μm thickness. The total number of microspheres found in section was determined for each animal. Data are mean values ± SEM expressed as number of microspheres per section of tissue. The clearance score, microspheres removed from brain, was calculated as the percent decrease in number of microspheres when compared to the control injection site. n = number of brain sections scored per group. Statistical analyses compared CSF injected sites with control sites.
[a] $p < 0.005$ by Student's t test using the Bonferroni correction.

(Giulian and Baker, 1986) or adult rat resident peritoneal mac-rophage (Table 1). Such observations support the idea that ame-boid microglia are a distinct class of mononuclear phagocyte more similar to bone marrow progenitor cells than to circulating blood monocytes. Although the origin of microglia remains un-certain, our findings are consistent with Rio-Hortega's hypoth-esis that primitive mesenchymal cells seed the embryonic brain with microglial precursors (Rio-Hortega, 1932).

Further study will be necessary to determine if factors similar to GM-CSF or multi-CSF are produced within the CNS. There are indications that the brain does contain CSF-like biologic activities. Our laboratory (D. Giulian, unpublished observa-tions), for example, has recently found 2 brain proteins that stimulate proliferation of ameboid microglia but not astroglia or oligodendroglia. Moreover, the greatest concentrations of these mitogens are recovered from brain tissue at periods of microglial proliferation. In vitro studies from Frei and Fontanna (1986) also suggest that a CSF-like biologic activity is produced in cell cultures of brain. The characterization of these factors and the identification of their cellular sources will help to elucidate mechanisms regulating the early phases of brain inflammation. Perhaps the local secretion of brain CSFs helps to control the growth of microglial populations during late embryonic devel-opment and at sites of injury.

The brain is often considered to be an immunologically priv-ileged site for it shows a delayed rejection of tissue grafts and normally has a very small population of antigen-presenting mononuclear phagocytes (Medawar, 1947; Head and Griffin, 1985). The in vivo application of CSFs to the brain stimulates a local inflammatory response by increasing both the number and effector capacities of mononuclear phagocytes. Similar ap-plication of immunomodulators may offer new opportunities to treat neurologic disease.

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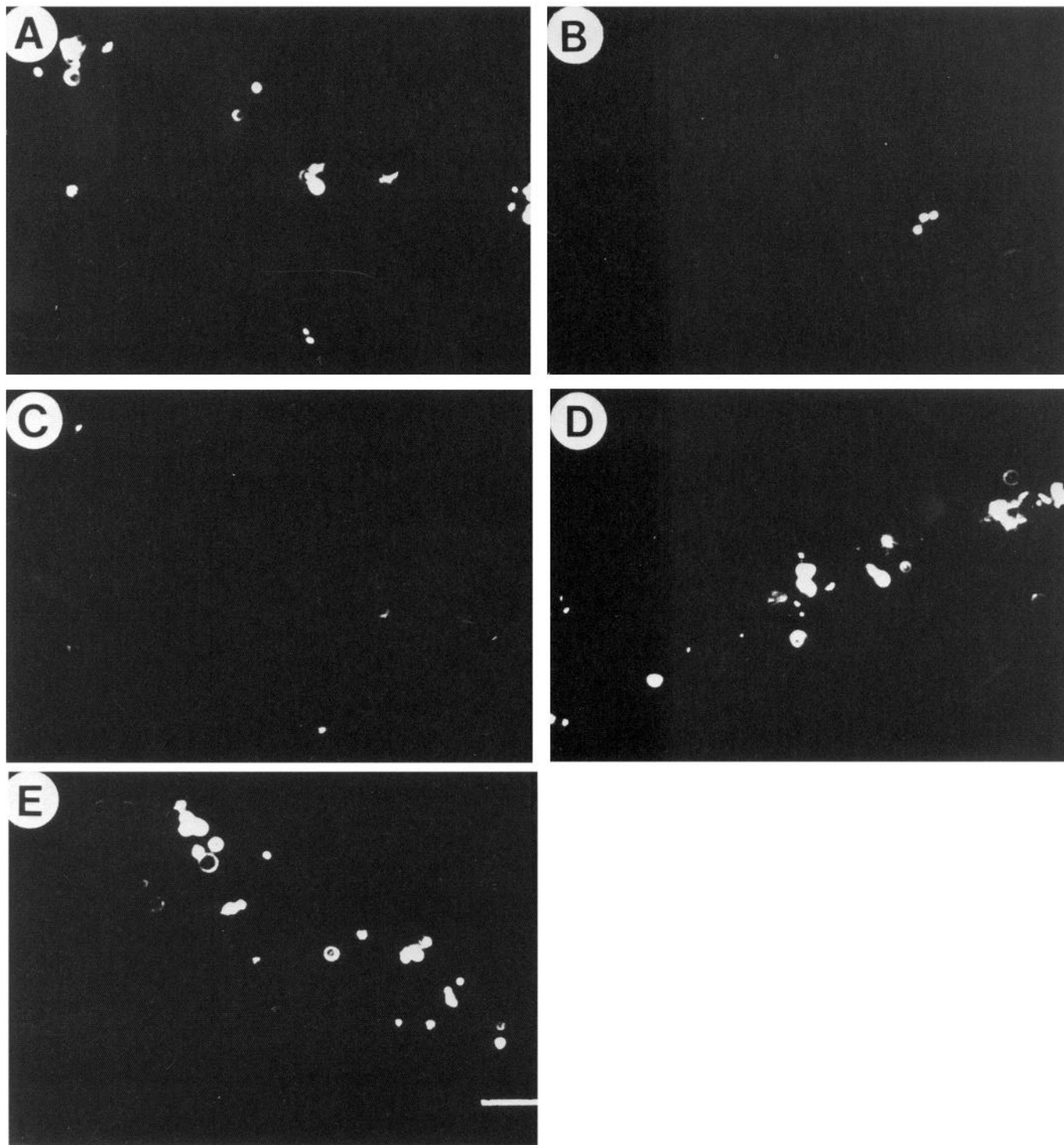
Figure 12. Fluorescence photomicrographs of microsphere clearance from the adult brain. Colony-stimulating factors were co-injected with fluorescently labeled microspheres into the cerebral cortex of adult rats. Clearance of the particles was examined 72 hr later. As shown here, there are fewer microspheres in those sites injected with 10 units of rm-GM-CSF (*B*) or 10 units of m-multi-CSF (*C*) compared with PBS controls (*A*), 50 units of rh-M-CSF (*D*), or 50 units of rh-G-CSF (*E*). Scale bar, 20 μm.

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