American Journal of Pathology, Vol. 143, No. 2, August 1993
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Short Communication

Interleukin-4 Down-Regulates Adult Human Astrocyte DNA Synthesis and Proliferation

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The T lymphocyte-derived cytokine, interleukin-4 (IL-4), was found to inhibit dose dependently basal DNA synthesis of cultured non-neoplastic human astrocytes isolated from epilepsy white matter tissue. The mitogenic effect of tumor necrosis factor on astrocytes was also inhibited by IL-4, and the inhibitory effect was abrogated by anti-IL-4 antibody but not by irrelevant IgG. Immunofluorescent analysis indicated significantly reduced numbers of glial fibrillary acidic protein-positive astrocytes incorporating nuclear bromodeoxyuridine in IL-4-treated cultures compared to control. These findings indicate that human adult astrocyte proliferation, in contrast to that reported for endothelial cells or fibroblasts, is sensitive to down-regulation by IL-4. (Am J Pathol 1993, 143:337–341)

Interleukin-4 (IL-4) is a pleiotrophic, T cell-derived cytokine.[1] Described initially as a B cell growth factor,[2] IL-4 has since been found to regulate a wide range of immune responses. IL-4 has been shown to enhance growth of T lymphocytes and mast cells,[3] augment cytolytic T cell activity,[4] down-regulate monocyte cytokine production,[5] and modulate major histocompatibility complex antigen expression in normal hematopoietic cells.[6]

The effects of IL-4 on astrocytes have not been established, to our knowledge, although astrocyte exposure to IL-4 may be hypothesized based upon immunohistochemical detection of T lymphocytes within the central nervous system during pathological conditions. In multiple sclerosis, for example, immunohistochemical studies from our laboratory and others have demonstrated abundant CD4+ and CD8+ T lymphocytes in the area of multiple sclerosis lesions.[7–10] Both CD4+ and CD8+ T cells have been shown to produce IL-4 in man; however, IL-4 production seems to be associated with a suppressor CD8+ subset, and with CD4+ T helper cells that regulate antibody synthesis.[11]

Several studies indicate that T cell-derived lymphokines can markedly alter the functional properties of normal astrocytes. Interferon-γ enhances class II major histocompatibility complex antigen expression on murine and human astrocytes[12,13] and has also been reported to stimulate *in vitro* astrocyte proliferation and *in vivo* gliosis.[14] Glial cell proliferation is also augmented by other T cell-derived products.[15] We demonstrate in this report that IL-4 down-regulates proliferation of adult human astrocytes, even in the presence of a mitogenic cytokine such as tumor necrosis factor (TNF). These data suggest that *in vivo*, IL-4 may counterbalance the stimulatory effects of inflammatory cytokines on astrocytosis.

Materials and Methods

Cell Culture

The astrocyte-rich, adult human glial cell line, W3N, was derived from temporal lobe white matter obtained during lobectomy for intractable epilepsy. Detailed characteristics have been previously de-

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Supported in part by a grant from the Epilepsy Foundation of America (MLE, BPB) and by NIH grant 1454-HL (MLE).

Accepted for publication April 17, 1993.

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scribed.[16] Experiments were carried out utilizing second through fourth passage cells from cryogenic storage.

Immunofluorescent Analyses

Cells were cultured on coverslips (75,000 cells/coverslip) and incubated for 48 hours with or without recombinant IL-4 (50 ng/ml, Genzyme, Cambridge, MA) in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2% fetal bovine serum, l-glutamine, and antibiotics (GIBCO, Grand Island, NY) (cRPMI). Cultures were incubated with 25 μmol/L bromodeoxyuridine (BrdU) for 24 hours before double-labeled immunofluorescent analysis as previously described.[17] After denaturation of native DNA, coverslips were incubated sequentially with rabbit anti-cow glial fibrillary acidic protein (GFAP), 1:50 (Dako, Carpinteria, CA); fluorescein isothiocyanate-conjugated goat anti-rabbit IgG, 1:20 (Kierkegaard and Perry, Gaithersburg, MD); and fluorescein isothiocyanate-conjugated anti-BrdU, 50 ug/ml (Boehringer-Mannheim, Indianapolis, IN). Coverslips were mounted in 90% glycerol, and 20 high-power fields (200×) per coverslip were examined under fluorescence microscopy by a single observer (MLE). Results were expressed as the percentage of GFAP(+) cells containing nuclear BrdU within each culture.

DNA Synthesis Assay

Determination of DNA synthesis by tritiated (3H) thymidine incorporation was carried out in a modification of methods previously described.[17] Cells were plated into microtiter plates at 5,000 cells/well in RPMI 1640 medium supplemented with 10% fetal bovine serum and cultured for 2 weeks. Cultures were then washed and reincubated for 24 hours in cRPMI alone, and cRPMI containing: IL-4 (0.1 to 100 ng/ml); TNF (10 ng/ml, Genzyme); or a combination of these reagents. Polyclonal rabbit antibody to IL-4 (Genzyme) or normal rabbit IgG (10 μg/ml) was included in some experiments. Four replicate wells were utilized per variable. Tritiated thymidine (5 uCi/ml) was included during the last 2 hours of culture. Cells were dispersed with trypsin and radioactivity was determined as previously described.[17] Results were expressed as mean-counts per minute (cpm) ± SD.

Proliferation Assay

Proliferation of cells in microtiter plate cultures was assessed as described previously by optical density measurement of crystal violet eluted from stained monolayers.[17] After 7-day incubation with or without test reagents, cultures were stained and results were expressed as mean optical density ± SD.

Statistical Analysis

Statistical significance of data from treated and control cultures was determined by two-tailed Student's *t*-test.

Results

DNA Synthesis

Basal DNA synthesis of culture W3N treated with IL-4 for 24 hours was inhibited in a dose-dependent manner, with 35 to 59% inhibition observed at 0.1 to 100 ng/ml IL-4 (Figure 1). In these experiments, astrocytes were cultured only in medium supplemented with 2% fetal bovine serum. To determine if IL-4 would antagonize an alternative mitogenic signal, cultures were treated with TNF in the presence and absence of IL-4 (Table 1). As we observed previously, 24-hour treatment with TNF elicited a burst of DNA synthesis.[17] Inclusion of IL-4 (10 ng/ml) significantly inhibited the effect of TNF by 73% ($P < 0.001$). The inhibitory effect of IL-4 on TNF stimulation was IL-4-dose-dependent (data not shown) and

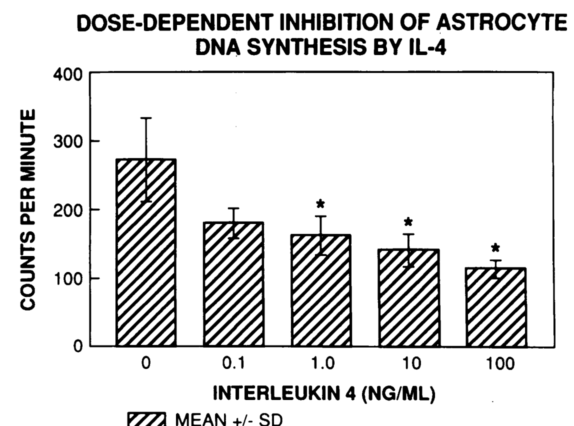

Figure 1. *Effect of IL-4 concentration on basal DNA synthesis of nonneoplastic astrocytes. Line W3N was cultured for 24 hours in the presence or absence of IL-4 at doses shown. Data represent mean counts per minute of tritiated thymidine incorporated during the last 2 hours of culture.* *$p < 0.025$ ($n = 4$) *compared to DNA synthesis in the absence of IL-4.*

Table 1. *IL-4 Inhibition of Astrocyte DNA Synthesis Elicited by TNF*

In vitro treatment	Counts per minute ± SD	Percentage inhibition
Medium	960 ± 134	
IL-4 (10 ng/ml)	520 ± 34	(46)* ($P < 0.001$)
TNF (10 ng/ml)	4,582 ± 543	
TNF + IL-4	1,221 ± 209	(73)† ($P < 0.001$)
TNF + IL-4 + anti-IL-4	3,861 ± 707	(16)† ($P > 0.05$)
TNF + IL-4 + normal rabbit IgG	2,316 ± 112	(50)† ($P < 0.001$)

* Compared to Medium.
† Compared to TNF. Line W3N was cultured for 24 hours with the reagents shown; tritiated thymidine was included during the last 2 hours of culture. Data represent mean counts per minute tritiated thymidine incorporated ($n = 4$).

was completely abolished by rabbit anti-IL-4 IgG but not normal rabbit IgG (Table 1).

Proliferation

Microtiter plate cultures exposed for 7 days to 10 ng/ml TNF demonstrated enhanced proliferation as previously described[17] (Figure 2). Inclusion of 10 ng/ml IL-4 in cultures significantly ($P < 0.001$) reduced proliferation, and this effect was abrogated by the presence of rabbit anti-IL-4 IgG but not normal rabbit IgG (Figure 2).

Immunofluorescence Analysis

To verify that affected cells were astrocytic, coverslip cultures of W3N were labeled with GFAP after 2-day exposure to IL-4 (50 ng/ml) or medium alone with inclusion of BrdU during the last 24 hours. The percentage of GFAP-positive astrocytes displaying nuclear BrdU was 12.3 ± 2.5% (SD) in cultures exposed to medium alone (Figure 3). In IL-4-treated cultures, the percentage of cells displaying both GFAP and BrdU was significantly ($P < 0.005$) reduced to 0.8 ± 0.8% ($n = 3$), indicating that DNA synthesis in GFAP-positive astrocytes was severely curtailed by IL-4 exposure.

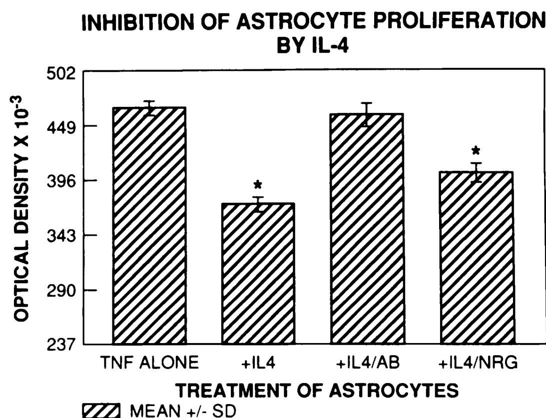

Figure 2. *Effect of IL-4 on TNF-stimulated proliferation of W3N astrocytes. Line W3N was cultured for 7 days in the presence of: TNF (10 ng/ml) alone; TNF + IL-4 (10 ng/ml); TNF + IL-4 + neutralizing antibody to IL-4 (AB) (10 ug/ml); and TNF + IL-4 + normal rabbit IgG (NRG) (10 ug/ml). Data represent mean optical density × 10-3; baseline optical density (237 ± 14) is that of cells cultured in medium alone. *p < 0.001 (n = 9) compared to TNF alone.*

Discussion

Gliosis, a condition characterized by astrocytic hypertrophy and proliferation, is a prominent pathological feature in numerous diseases affecting the central nervous system. Although mechanisms contributing to gliosis are still inadequately understood, evidence suggests that specific cytokines may contribute to this process. IL-1,[18] TNF,[19] and interferon-γ[14] all have been reported to stimulate gliosis *in vivo* as well as astrocyte proliferation *in vitro*.[14,17,18,20] Our data suggest that IL-4, which inhibits astrocyte proliferative responses *in vitro* may also inhibit gliosis *in vivo*. Confirmation of this speculation must await additional study, however.

IL-4 has been observed to down-regulate proliferation of IL-2-stimulated normal B lymphocytes[21] and lymphokine-activated killer cells.[22] In addition, IL-4 has been shown to reduce growth of several types of human tumor cell lines *in vitro*.[23,24] In contrast, IL-4 stimulates growth of endothelial cells[25] and fibroblasts;[26] moreover, the mitogenic effect of TNF on human foreskin fibroblasts has been reported to be unaffected by IL-4.[24]

Our findings suggest that IL-4 regulates proliferation in a cell-specific manner that differentiates astrocytes from endothelial cells or fibroblasts. Even in the presence of a potent mitogenic signal like TNF, we observed that IL-4 significantly down-regulated astrocyte DNA synthesis and proliferation. The mechanisms involved in this selective effect of IL-4 on astrocytes are currently under investigation. These data suggest, however, that within the central nervous system, IL-4 may serve to attenuate the effects of other stimulatory cytokines on astrocyte proliferation.

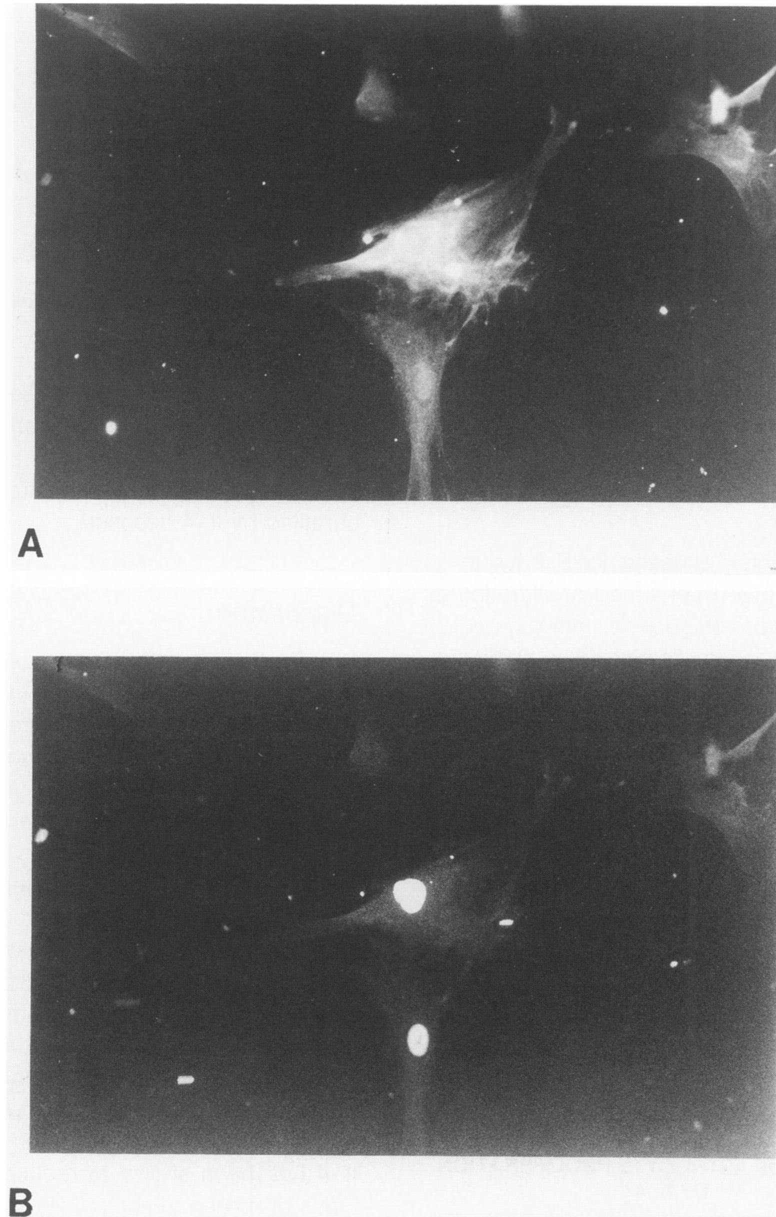

Figure 3. *Immunohistochemical detection of GFAP and BrdU. Photomicrographs of astrocytic cells in line W3N cultured in medium alone, demonstrating the presence of:* **(A)** *cytoplasmic GFAP and* **(B)** *nuclear incorporation of fluorescent BrdU (200×).*

Acknowledgment

Special thanks to Mrs. Alice Kiffer for help in preparation of the manuscript.

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