Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9221–9225, December 1986
Neurobiology

Interleukin 2 mediates the inhibition of oligodendrocyte progenitor cell proliferation *in vitro*

(glia/growth regulation/lymphokine/anti-Tac antibody receptor)

RUSSELL P. SANETO*, AMNON ALTMAN†, ROBERT L. KNOBLER‡, HOWARD M. JOHNSON§, AND JEAN DE VELLIS*¶

*Department of Anatomy and Psychiatry, UCLA School of Medicine, Mental Retardation Research Center, Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles, CA 90024; †Scripps Clinic and Research Foundation, La Jolla, CA 92037; ‡Department of Neurology, Thomas Jefferson Medical College, Philadelphia, PA 19107; and §Department of Comparative and Experimental Pathology, University of Florida, Gainesville, FL 32610

Communicated by Elizabeth F. Neufeld, July 15, 1986

ABSTRACT **In the immune system, T-lymphocyte proliferation depends on interleukin 2 [IL-2 (T-cell growth factor)] interaction with specific receptors. In this study we show that IL-2 can specifically inhibit the proliferation of neonatal rat oligodendrocyte progenitor cells cultured in a serumless, chemically defined medium (oligodendrocyte-defined medium; ODM). IL-2 inhibited both [^{3}H]thymidine incorporation and increase in cell number. Specificity was shown by precipitating IL-2 activity with anti-IL-2 antiserum. Furthermore, growth inhibition depended on the expression of Tac (an anti-IL-2 receptor monoclonal antibody)-positive receptors (IL-2 receptor). When cells were cultured in the presence of IL-2, both Tac-positive staining and growth inhibition were no longer expressed. The addition of interleukin 1 had no effect on [^{3}H]thymidine incorporation or changes in cell number. However, when IL-1 was subsequently added together with IL-2, Tac expression and IL-2-mediated inhibition of cell proliferation was induced. This inhibitory effect was not due to a sensitive subpopulation because >90% of the culture was Tac positive. Taken together, these data show that IL-2 can specifically inhibit oligodendrocyte proliferation and acts via Tac-positive receptors.**

A growing body of data suggests that bidirectional communication can exist between the immune and central nervous system (for review, see ref. 1). In addition to the potential involvement of neuroendocrine influence on immune function (2, 3), there are also reports of immunoregulation of brain activity (4, 5). The most striking examples of the latter condition are in patients with immune-mediated neurological disorders such as multiple sclerosis. The pathological description of axonal demyelination and glial hyperplasia in multiple sclerosis suggests the intimate involvement of T-cells (6–8), macrophages (9–11), and possibly their secreted peptide hormones (12).

The direct immunoregulation of central nervous system function by immune system growth factors has been shown only recently. Increased leukocyte number in demyelinated areas provides within the central nervous system a source of interleukin 1 (IL-1), interleukin 2 (IL-2), and γ interferon (11, 12). *In vivo* and *in vitro* studies have shown specific interaction with brain cells: γ interferon induces both H-2 (class I major histocompatibility antigen) and Ia antigen (class II major histocompatibility antigen) on astrocytes and H-2 antigen on oligodendocytes and neurons (10); IL-1 can both be synthesized by and act as a mitogen for astrocytes (13, 14); and products produced by the human T-cell line Mo can induce IL-2 receptors, as detected by the anti-IL-2 receptor-monospecific antibody anti-Tac, on oligodendrocytes and induce glial cell proliferation (15). However, several studies have shown that IL-2 has no effect on oligodendrocyte proliferation (14, 15). These studies were carried out in serum with culture conditions where the capacity of isolated oligodendrocytes to proliferate is minimal (16, 17).

Recently, we developed a serum-free chemically defined medium (oligodendrocyte-defined medium; ODM) that supports the rapid proliferation of immature oligodendrocytes purified from primary rat cerebral cultures (17). The purified cell population is 95–98% homogeneous with respect to the oligodendrocyte marker, glycerol phosphate dehydrogenase, and the glial progenitor cell marker, the mouse monoclonal antibody A2B5, but largely lacks galactocerebroside and myelin basic protein. Since the immunological marker galactocerebroside is now generally used as a criterion for the identification of oligodendrocytes, we find it useful for purpose of clarification to call the preparation of A2B5/glycerol phosphate dehydrogenase-positive cells "oligodendrocyte progenitor cells." We feel this is justified because, under the conditions of ODM, these cells give rise in 5 days to cells identified as oligodendrocytes by biochemical, immunological, and ultrastructural criteria (17).

In the present report, we demonstrate that the T-cell growth factor IL-2 (18, 19) can modulate the proliferation of oligodendrocyte progenitor cell cultured in ODM. IL-2 inhibits both [^{3}H]thymidine incorporation and increase in cell number. This action of IL-2 was specifically mediated by Tac-positive receptors.

MATERIALS AND METHODS

Cell Culture. Purified cultures of rat oligodendrocyte progenitor cells were prepared as modified (17) and seeded in Falcon plastic 24-well culture plates at a density of 10^5 cells per well. The culture medium was Dulbecco–Vogt-modified Eagle's medium/Ham's F-12 medium, 1:1 (vol:vol), with 1.2 g of $NaHCO_3$ per liter and 15 mM Hepes buffer (serum-free medium; SFM) supplemented with 10% (vol/vol) fetal calf serum. SFM was routinely made with pyrogen-free, ultrapure distilled water.

ODM. ODM consisted of SFM, pyruvate (110 μg/ml), insulin (5 μg/ml), transferrin (500 ng/ml), and pituitary fibroblast growth factor (FGF; 75 ng/ml).

Cell Proliferation. Purified oligodendrocyte progenitor cells were initially seeded in culture medium. After 18–24 hr of incubation to allow attachment, cells were washed three

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ODM, oligodendrocyte-defined medium; IL-1 and IL-2, interleukins 1 and 2; FGF, fibroblast growth factor; SFM, serum-free medium.

¶To whom reprint requests should be addressed at: Neurobiochemistry Group, MRRC/NPI, 760 Westwood Plaza, Room 68-177, Los Angeles, CA 90024.

times with SFM and ODM with or without experimental supplements added. Cells were counted in an aliquot, and this cell density was used as the actual seeding density. Cells were subsequently cultured and counted as described (17). There was never evidence of cell death or debris in either control or experimental cultures.

[^{3}H]Thymidine incorporation was estimated from trichloroacetic acid-precipitated material. Briefly, oligodendrocytes were cultured in ODM with and without experimental supplements for 22 hr and subsequently were exposed to 1.0 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine per ml for 2 hr. Cultures were then processed and assayed for radioactivity as described (20).

Immunocytochemical Staining. For immunocytochemical staining, cells were cultured on glass coverslips and incubated with either anti-Tac antibody or control sera at a dilution of 1:5000 in phosphate-buffered saline (0.03 M phosphate buffer/0.9% NaCl, pH 7.4) for 1–2 hr at room temperature. Cells were rinsed three times in phosphate-buffered saline and fixed in 2% paraformaldehyde (wt/vol) for an additional 2 hr. After the phosphate-buffered saline washes were repeated, cells were incubated with biotin-conjugated goat anti-mouse IgG at a dilution of 1:125 in phosphate-buffered saline for 16–18 hr at 4°C. Subsequently, cultures were rinsed three times and incubated with tetranitroblue tetrazolium in the dark for 15 min at room temperature. The stained cultures were then rinsed, mounted, and photographed.

IL-2 Bioassay. IL-2 bioactivity of both purified astrocyte- and oligodendrocyte-conditioned media was determined by its ability to induce [^{3}H]thymidine incorporation in the murine IL-2-dependent CTLL-2 cell line (21). Purified astrocytes (22) were grown to confluence in culture medium. Cultures were then washed three times with SFM and cultured for an additional 48 hr in SFM. The conditioned medium was then processed as described (17). Purified oligodendrocytes were cultured in ODM for 48 hr, and the conditioned medium was processed similar to astrocyte-conditioned medium.

In all experiments, one unit of IL-2 activity—0.12 ng/ml as determined for recombinant IL-2—gives 50% of maximum proliferation in this assay and is equivalent to 1.5 IL-2 "Reference Reagent units" as defined by the Biological Response Modifiers Program of the National Cancer Institute. Dose–response experiments of both conditioned media were performed, and no IL-2 activity was detected.

IL-2. Human IL-2 (natural IL-2) was prepared from the supernatant of the Jurkat culture medium as described by Robb *et al.* (23) and purified by using a rabbit anti-human recombinant IL-2 immunoaffinity column. By gel electrophoresis, this IL-2 preparation appeared homogeneous. Recombinant IL-2 was a gift from Cetus (Emeryville, CA).

Materials. Unless otherwise stated, all chemicals were purchased from Sigma. Pituitary FGF was purified by a modification (20) of the procedure of Gospodarowicz (24). Ham's F-12 medium (H-17), Dulbecco's modified Eagle's medium (H-12), Hanks' balanced salt solution (Mg^{2+}/Ca^{2+}-free), and fetal calf serum were obtained from Irvine Scientific (Irvine, CA). Trypsin (1% Enzar-T) was from Reheis (Kankakee, IL), protein A-Sepharose was from Pharmacia, and IL-1 was from Collaborative Research (Waltham, MA). The monoclonal antibody to the Tac-positive receptor was a generous gift of T. Waldman (National Institutes of Health).

RESULTS

Isolated rat oligodendrocyte progenitor cells induced to proliferate when grown in ODM have been characterized biochemically, ultrastructurally, and immunologically (17). After 5 days of growth, routine cultures are 95–98% oligodendrocytes with 2–5% of the cells staining positive for glial fibrillary acid protein (astrocytes) and none for nonspecific esterase-positive cells (macrophages).

Table 1. IL-2 inhibition of [^{3}H]thymidine incorporation into oligodendrocyte progenitor cells

Medium	Treatment	cpm ± SD
	Treatment added at day 1	
ODM	None	2365 ± 88
	IL-1	2250 ± 100
	IL-2	1636 ± 197*
	IL-1/IL-2	1790 ± 105*
SSM	None	260 ± 15
	IL-1	254 ± 16
	IL-2	190 ± 21†
	IL-1/IL-2	211 ± 10†
	Treatment added at day 3	
ODM	None	2840 ± 234
	IL-1	2434 ± 316
	IL-2	1588 ± 390*
	IL-1/IL-2	1989 ± 185*
SSM	None	439 ± 119
	IL-1	449 ± 101
	IL-2	233 ± 55
	IL-1/IL-2	350 ± 102

The method of detection of [^{3}H]thymidine incorporation is given in the text. Treatments are added at either the initial medium change to defined medium (day 1) or at the next medium change (day 3). IL-2 was used at 5 units per ml, and IL-1 was used at 3 units per ml; cpm represent the mean of three cultures ± SD. The results are representative of triplicate experiments. SSM, serum-supplemented medium.
*Significant at $P < 0.005$.
†Significant at $P < 0.01$.

IL-2 Inhibition of [^{3}H]Thymidine Incorporation. IL-2 inhibited incorporation of [^{3}H]thymidine in oligodendrocyte progenitor cells cultured in both ODM and serum-supplemented medium (Table 1). IL-2 was active over a wide range of concentrations (Fig. 1). Affinity-purified IL-2 and recombinant IL-2 dosage was examined (data not shown).

To examine whether IL-2 could inhibit thymidine incorporation in cells in logarithmic-phase growth, purified oligodendrocyte progenitor cells were allowed to proliferate for 2

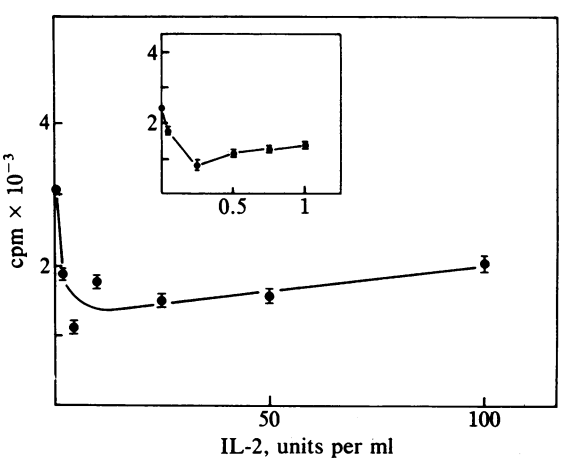

FIG. 1. Dose–response curve of [^{3}H]thymidine incorporation into oligodendrocyte progenitor cells as a response to IL-2. Oligodendrocyte progenitor cells were isolated and cultured for 24 hr in ODM containing varying amounts of IL-2 (affinity-purified). Radiolabel was added 2 hr before termination of the experiment. Cultures were processed and assayed for radioactivity as described in the text. (*Inset*) Data derived from low concentrations of IL-2. Both graphs represent three experiments, and data points are means of triplicate trials ± SD.

Table 2. The precipitation of Il-2 activity with specific antiserum against IL-2

Condition	cpm ± SD
None	700 ± 75
IL-2	402 ± 50*
IL-2/anti-IL-2 antiserum	637 ± 57
IL-2/preimmune serum	385 ± 35*

IL-2 activity is based on [^{3}H]thymidine incorporation into acid-precipitated material as described in the text. IL-2 was used at 5 units per ml. IL-2 was allowed to interact with anti-IL-2 antiserum for 18 hr at 4°C, at which time protein A-Sepharose was added. After 6 hr at 4°C, the solution was centrifuged in a Microfuge for 2 min before added to the bioassay system. In the control experiments, either sterile saline or preimmune serum was substituted for IL-2 or anti-IL-2 antiserum. In this experiment, cells were seeded at 5×10^4 cells per ml; cpm represents the mean of three cultures ± SD. This experiment is representative of duplicate experiments.
*Significant at $P < 0.005$.

days in ODM. Then the medium was changed, and IL-2 was added for 24 hr. IL-2 inhibited the incorporation of [^{3}H]thymidine (Table 1). IL-2 was also active on 5-day-old cultures (data not shown).

The specificity of IL-2 action on oligodendrocyte progenitor cells was ascertained in two ways. First, IL-2 had no effect on purified cultures of astrocytes in the [^{3}H]thymidine incorporation assay (data not shown), indicating that contaminating astrocytes did not affect the thymidine incorporation data. Second, monospecific antibodies to IL-2 (25) abrogated IL-2 activity on oligodendrocytes, whereas preimmune serum had no effect (Table 2).

IL-2 Inhibition of Cell Proliferation. The exclusive use of [^{3}H]thymidine incorporation data to define modulation of actual cell numbers may lead to false conclusions (26). To substantiate [^{3}H]thymidine incorporation data, we performed cell-growth experiments over a 5-day period (Fig. 2). Added by itself, IL-2 had no effect on cell growth. After 5 days of culture, those cultures with IL-2 present during the entire 5 days had 3.46 (± 0.20) $\times 10^5$ cells, whereas those cultures without IL-2 present had a cell count of 3.62 (± 0.27) $\times 10^5$ cells. However, in combination with IL-1, IL-2 significantly inhibited cell proliferation (Fig. 2). By itself, added IL-1 had no effect on cell number (data not shown).

IL-1 Regulation of Anti-Tac-Positive Receptors. Tac, an anti-IL-2 receptor monoclonal antibody (27, 28), was used to

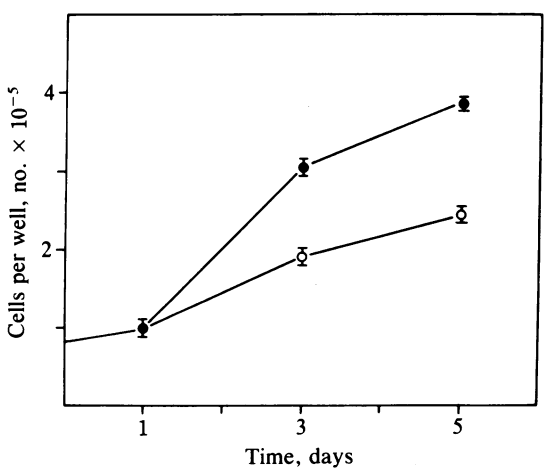

FIG. 2. Growth curve of isolated oligodendrocyte progenitor cells as a response to IL-1 combined with IL-2. Oligodendrocyte progenitor cells were isolated and cultured in ODM with (○) or without (●) IL-1 (3 units per ml) and IL-2 (2 units per ml). Data points represent the cell number per well; values are means of six wells ± SEM. This is representative of duplicate experiments.

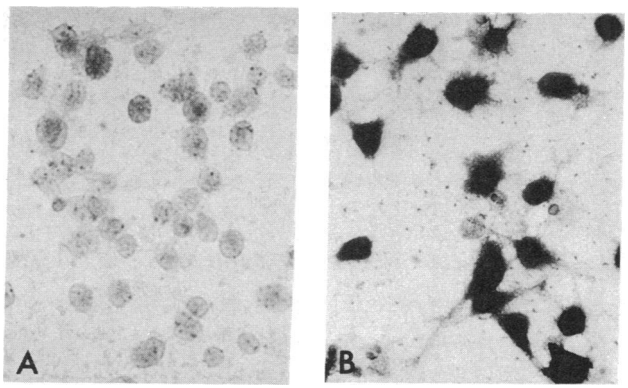

FIG. 3. Immunocytochemical staining of pure isolated oligodendrocyte progenitor cells with anti-Tac monoclonal antibody. Micrographs are bright-field photographs of the avidin–biotin complex, with glucose oxidase staining of oligodendrocytes after 24 hr in ODM. (*A*) Micrograph of oligodendrocytes stained with control sera. (*B*) Sister culture stained with anti-Tac (1:5000). (×270.)

investigate whether IL-2 effects might be mediated by specific receptors as described on lymphocytes (23). Tac-positive receptors were found on >90% of the purified oligodendrocyte progenitor cells at the first medium change to ODM (Fig. 3). When the cultures were continuously exposed to IL-2 for 5 days in ODM, none of the cells expressed Tac-positive receptors. However, when the cells were not exposed to IL-2 or when IL-1 had been added alone or together with IL-2 for 5 days, >90% of the surface decoration varied greatly (Fig. 4).

IL-1 Modulation of IL-2 Inhibition of [^{3}H]Thymidine Incorporation. IL-1 addition to cultures switched to ODM had no effect on thymidine incorporation by itself nor enhanced IL-2 inhibition (Tables 1 and 3). After 48 hr of IL-2 treatment, no significant effect on [^{3}H]thymidine incorporation was observed (Table 3). However, in sister cultures with added IL-1, IL-2 inhibited thymidine incorporation (Table 3). This effect was also found at 5 days in culture (data not shown). Furthermore, the addition of IL-1 had no effect on cell proliferation but induced Tac-positive receptors. The IL-2 inhibition of [^{3}H]thymidine incorporation was only expressed under conditions of Tac receptor expression: (*i*) cultured oligodendrocytes not previously exposed to IL-2 (Table 1 and Figs. 3 and 4) and (*ii*) where IL-1 was present (Table 3 and Fig. 4). These data suggest the effect of IL-2 can be mediated via IL-1 modulation of Tac-positive receptors.

DISCUSSION

IL-2 is a lymphokine synthesized and secreted by some T cells after activation with antigen or mitogen in the presence of IL-1 (29, 30). The present study demonstrates that IL-2 specifically inhibits the proliferation of isolated oligodendrocyte progenitor cells in culture. Specificity was demonstrated by using homogeneous natural IL-2 and recombinant IL-2 and precipitating activity with specific antibodies to IL-2. Both [^{3}H]thymidine incorporation and cell number are reduced in the presence of IL-2. This inhibitory effect was only found when oligodendrocyte progenitor cells expressed the Tac receptor, suggesting a specific receptor–ligand-induced response.

To exert a biological effect on T cells, IL-2 must interact with specific high-affinity membrane receptors (23, 31). On T cells, IL-2 receptors are expressed only after activation by an antigen, mitogen, or anti-clonotype antibody in the presence of IL-1 (32, 33). Once activated, receptor expression is transient and lasts no longer than a few cell divisions (32, 33). The monoclonal antibody anti-Tac is specific for IL-2 recep-

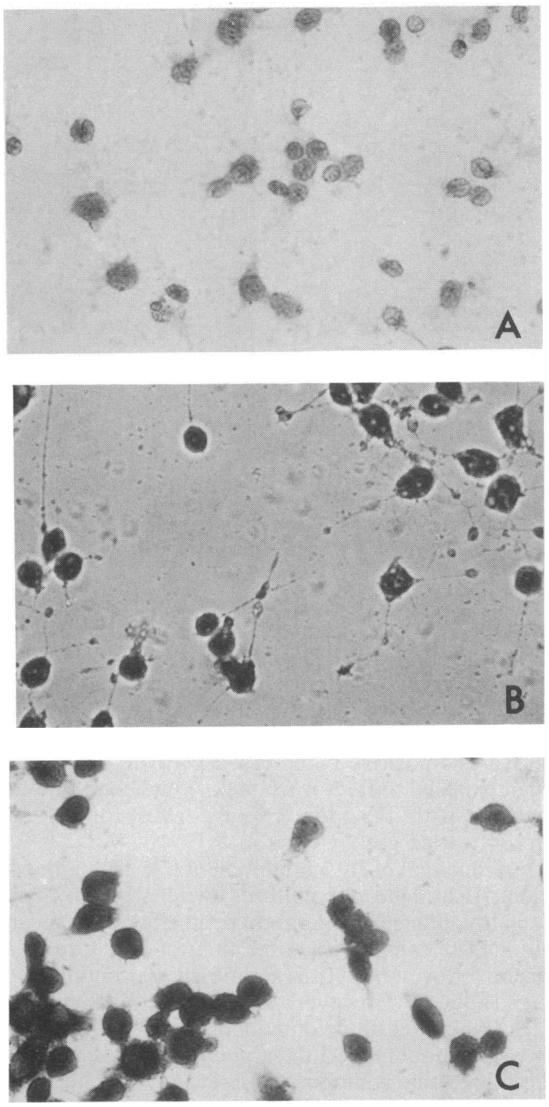

FIG. 4. Immunocytochemical staining of isolated oligodendrocytes with anti-Tac monoclonal antibody after 5 days in culture. Micrographs are of the avidin–biotin complex, with glucose oxidase staining of pure oligodendrocytes cultured for 5 days, and utilize bright-field optics. (*A*) Culture grown in the presence of IL-2 (2 units per ml) and stained with anti-TAC antibody. (*B*) Parallel cultures grown in the presence of both IL-1 (3 units per ml) and IL-2 (2 units per ml) and immunocytochemically stained with anti-Tac antibody. (*C*) Parallel cultures grown in ODM alone and stained with the anti-Tac antibody. (×330.)

tors on lymphocytes (23, 27). Oligodendrocytes isolated and cultured in ODM express Tac-positive receptors throughout the 5-day culture period. However, in the continued presence of IL-2, the Tac-positive receptor disappeared. Furthermore, the inhibition of [^{3}H]thymidine incorporation was observed only when the Tac receptor was expressed. Although it cannot be ruled out that the anti-Tac antibody may be interacting with a common epitope on another protein, the modulation of IL-2 activity by the expression of the Tac-positive protein strongly suggests that the oligodendrocyte Tac receptor is the IL-2 receptor, or at least very similar to the IL-2 receptor. The fact that picomolar IL-2 concentrations inhibited oligodendrocyte-progenitor-cell proliferation suggests that the effect of IL-2 is mediated by high-affinity receptors because only such receptors would respond to such IL-2 concentrations. A recent study (31) showed that only high-affinity IL-2 receptors were internalized upon IL-2 binding. Thus, the down-regulation of oligodendrocyte Tac-positive receptor may also reflect the receptor high-affinity status. However, the exact affinity of this receptor remains to be determined.

Table 3. IL-1 modulation of IL-2 inhibition of [^{3}H]thymidine incorporation into oligodendrocyte progenitor cells previously exposed to IL-2

Treatment	cpm ± SD
None	2360 ± 86
IL-1	2010 ± 150
IL-2	1850 ± 194
IL-1/IL-2	1260 ± 132*

Oligodendrocytes were prepared and seeded as described in the text. Both IL-1 (3 units per ml) and IL-2 (5 units per ml) were added at the initial medium change and at the medium change at day 3. At this time cultures were assayed for thymidine incorporation as described in the text; cpm represents the mean of three cultures ± SD. Experimental data shown are representative of three such experiments.
*Significant at $P < 0.005$.

Recently, Meuer *et al.* (34) have shown that some normal activated T cells may be induced by IL-2 to express IL-2 receptors. In the presence of IL-2, Tac-positive receptors on oligodendrocytes were down-regulated. The addition of IL-1 was found to induce the expression of Tac-positive receptors, even in the presence of IL-2. This suggests that IL-1 is regulating, at least in part, the expression of IL-2 receptors on oligodendrocytes. Conditioned media from purified populations of astrocytes and oligodendrocytes were not found to contain IL-2-like activity. Thus, it seems unlikely that an IL-2-like activity from the culture system could be affecting Tac protein expression. In T-cell stimulation, IL-1 induction of IL-2 receptors requires a costimulator (29, 30). What the costimulator would be in our system is presently unknown. Another intriguing finding is the initial expression of Tac-positive receptors. Prior to isolation, oligodendrocytes are grown together with astrocytes in the presence of serum. Fontana *et al.* (35) and Giulian and Lachman (14) have found that, given the proper stimulator, astrocytes can secrete IL-1. Together with a component(s) in serum, the astrocyte-derived IL-1 may induce the expression of IL-2 receptors. However, at this time we cannot speculate what the initial Tac-positive-receptor inducer might be. Although IL-2 interaction with its specific receptor induces proliferation of resting T cells, this interaction can also give negative growth signals to T cells (36). In our study, this ligand–receptor interaction partially inhibited oligodendrocyte-progenitor-cell proliferation. This partial inhibition is probably not the result of IL-2 action on a subpopulation of cells, since IL-2 receptors are expressed on almost the entire population of cells and this population has been shown to be proliferative (17). In addition, IL-2 inhibition of [^{3}H]thymidine incorporation was not the result of cytotoxicity effects. There was never a difference of cell degeneration or death between treated and untreated cells. Furthermore, the specificity of IL-2 action in concert with its receptor expression on oligodendrocytes strongly suggests a peptide-mediated physiological effect on oligodendroglial function.

Several studies indicate that cells of the central nervous system can respond to and produce mediators of immune function. Astrocytes have been shown to produce IL-1 (14, 35) and to proliferate upon exposure to IL-1 (14). Peptides released from ameboid microglia after trauma induce proliferation of cultured astrocytes (37). Oligodendrocytes proliferate in response to a growth factor derived from supernatants of a transformed human T-cell line, Mo (15, 38). However, two recent studies indicated that IL-2 had no effect on oligodendrocytes (14, 15). In one study (14) IL-2 had no

effect on cell number when oligodendrocytes were cultured in serum-containing medium. Our data indicate that IL-1 needs to be present for changes in cell number to occur. The other study found no mitogenic effects of IL-2 on galactocerebroside-positive oligodendrocytes (15). Recently, Temple and Raff (39) have shown that oligodendrocytes cultured from rat optic nerve may cease to proliferate upon expressing galactocerebroside. The maturity of the oligodendrocyte may account for lack of an IL-2 effect. The population of oligodendrocytes used in this study are developmentally less mature (17). They rapidly undergo proliferation and express the progenitor cell marker A2B5. The marker A2B5 has been shown to be expressed on progenitor cells that can eventually express galactocerebroside (39, 40). Under our defined conditions, the proliferating cell type does not express the astrocyte marker protein glial fibrillary acidic protein (40). Furthermore, this cell expresses the oligodendrocyte marker glycerol phosphate dehydrogenase and its induction by hydrocortisone (41) and later expresses galactocerebroside and myelin basic protein (17, 42). This would suggest that IL-2 is acting on an immature oligodendrocyte or oligodendrocyte progenitor cell.

The data presented show that IL-2 can specifically inhibit the proliferation of isolated oligodendrocyte progenitor cells. This action of IL-2 is mediated by expression of Tac-positive receptors. The specificity of lymphokine with its receptor on oligodendrocyte progenitor cells suggests the potential of immunoregulation of oligodendrocyte physiology. In the context of potential remyelination by oligodendrocytes, limited remyelination occurs in immune-mediated demyelination, whereas in other conditions, such as lysolecithin-induced demyelination (43), remyelination readily occurs. The extrapolation to our cell culture observations to *in vivo* leads us to speculate that lymphokines in a lesion can affect proliferative potential of astrocytes and oligodendrocytes differentially and, thus, have an impact on whether gliosis and scar formation or remyelination and repair predominate. Thus, the further characterization of IL action on oligodendrocyte function may reveal some of the mechanisms involved in immune system mediation of central nervous system diseases such as multiple sclerosis.

The authors thank Michele Bloom for preparation of the manuscript and Francesco Chiappelli for technical assistance. This work was supported by National Institutes of Health Grants HD06576, CA35299, and AM35411; Department of Energy Contract DE-AC03-76-00012; Basic Research Support Grant from the University of California, Los Angeles, School of Medicine; National Multiple Sclerosis Society Grant RG-722-3; a Leukemia Society Scholarship to A.A.; and Teacher Investigator Development Award NS00961 and Mary L. Smith Charitable Lead Trust to R.L.K.

1. Besedovsky, H. O., del Rey, A. E. & Sorkin, E. (1983) *Immunol. Today* **4**, 342–346.
2. Gilman, S. C., Schwartz, J. M., Milner, R. J., Bloom, F. E. & Feldman, J. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4226–4231.
3. Johnson, H. M., Smith, E. M., Torres, B. A. & Blalock, J. E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4171–4174.
4. Blalock, J. E., Harbour-McMenamin, D. & Smith, E. M. (1985) *J. Immunol.* **135**, 858s–861s.
5. Oppenheim, J. J. & Gery, I. (1982) *Immunol. Today* **3**, 113–119.
6. Sandberg-Wollheim, M. (1983) *Scand. J. Immunol.* **17**, 575–581.
7. Traugott, U., Reinherz, E. L. & Raine, C. S. (1983) *Science* **219**, 308–310.
8. Raine, C. S., Barnett, L. B., Brown, A., Behar, T. & McFarlin, D. E. (1980) *Lab. Invest.* **43**, 150–157.
9. Williams, K., Hart, D., Fabre, J. & Morris, P. (1986) *Transplantation* **29**, 274–279.
10. Wong, G. H. W., Bartlett, P. F., Clark-Lewis, I., Battye, F. & Schrader, J. W. (1984) *Nature (London)* **310**, 688–691.
11. Prineas, J. W., Kwon, E. E., Sternberger, N. H. & Lennon, V. A. (1984) *J. Neuroimmunol.* **6**, 251–264.
12. Waksman, B. (1985) *Nature (London)* **318**, 104–105.
13. Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. & Weber, E. (1982) *J. Immunol.* **129**, 2413–2419.
14. Giulian, D. & Lachman, L. B. (1985) *Science* **228**, 497–499.
15. Merrill, J. E., Kutsunai, S., Mohlstrom, C., Hofman, F., Groopman, J. & Golde, D. W. (1984) *Science* **224**, 1428–1430.
16. Szuchet, S., Stefansson, K., Wollman, R. L., Dawson, G. & Aronson, B. G. W. (1980) *Brain Res.* **200**, 151–164.
17. Saneto, R. P. & de Vellis, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3509–3513.
18. Robb, R. J. (1984) *Immunol. Today* **5**, 203–209.
19. Smith, K. A. (1984) *Annu. Rev. Immunol.* **2**, 319–333.
20. Saneto, R. P. & de Vellis, J. (1985) *Dev. Neurosci.* **7**, 340–350.
21. Smith, K. A., Favata, M. F. & Oroszlan, S. (1983) *J. Immunol.* **131**, 1808–1815.
22. McCarthy, K. D. & de Vellis, J. (1980) *J. Cell Biol.* **85**, 890–902.
23. Robb, B. M., Munck, A. & Smith, K. A. (1981) *J. Exp. Med.* **154**, 1455–1474.
24. Gospodarowicz, D. (1975) *J. Biol. Chem.* **250**, 2515–2520.
25. Cardenas, J. M., Marshall, P., Henderson, B. & Altman, A. (1986) *J. Immunol. Methods*, in press.
26. Mauer, H. R. (1981) *Cell Tissue Kinet.* **14**, 111–120.
27. Leonard, W. J., Depper, J. M., Robb, R. J., Waldman, T. A. & Greene, W. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6957–6961.
28. Uchiyama, T., Broder, S. & Waldman, T. A. (1981) *J. Immunol.* **126**, 1393–1397.
29. Wagner, H., Hardt, C., Heeg, K., Pfizenmaier, K., Solbach, W., Bartlett, R., Stockinger, H. & Roffinghoff, M. (1980) *Immunol. Rev.* **51**, 215–256.
30. Lafferty, K. J., Andrus, L. & Prowse, S. J. (1980) *Immunol. Rev.* **51**, 279–314.
31. Weissman, A. M., Harford, J. B., Svetlik, P. B., Leonard, W. L., Depper, J. M., Waldman, T. A., Greene, W. C. & Klausner, R. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1463–1466.
32. Kaplan, D. R., Braeiale, V. L. & Braciale, T. J. (1984) *J. Immunol.* **133**, 1966–1969.
33. Hemler, M. E., Brenner, M. B., McLean, J. M. & Strominger, J. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2172–2175.
34. Meuer, S. C., Hussey, R. E., Cantrell, D. A., Hodgdon, J. C., Schlossman, S. F., Smith, K. A. & Reinherz, E. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1509–1513.
35. Fontana, A. B., Kristensen, F., Dubs, R., Gemsa, D. & Weber, E. (1982) *J. Immunol.* **129**, 2413–2419.
36. Hatakeyama, M., Minamoto, S., Uchiyama, T., Hardy, R. R., Yamada, G. & Taniguchi, T. (1985) *Nature (London)* **318**, 467–470.
37. Giulian, D. & Baker, T. J. (1985) *J. Cell Biol.* **101**, 2411–2415.
38. Benveniste, E. N., Merrill, J. E., Kaufman, S. E., Golde, D. W. & Gasson, J. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3930–3934.
39. Temple, S. & Raff, M. C. (1986) *Cell* **44**, 773–779.
40. Raff, M. C., Miller, R. H. & Noble, M. (1983) *Nature (London)* **303**, 390–396.
41. Leveille, P. J., McGinnis, J. E., Maxwell, D. S. & de Vellis, J. (1980) *Brain Res.* **196**, 287–305.
42. Abney, E. R., Bartlett, P. P. & Raff, M. C. (1981) *Dev. Biol.* **83**, 301–310.
43. Arenella, L. S. & Herndon, R. M. (1984) *Arch. Neurol.* **41**, 1162–1165.