Proliferation of Astroglia and Oligodendroglia in Response to Human T Cell–Derived Factors

Abstract. *Human T lymphocytes transformed by human T cell leukemia-lymphoma viruses or activated by lectins were found to produce stimulating factors that promoted both proliferation and maturation of oligodendroglial and astroglial cells in vitro.*

Mature oligodendrocytes can proliferate in vivo after trauma to the central nervous system (*1*). Oligodendroglial hypercellularity, astrocytic proliferation, and gliosis are seen in plaques and surrounding white matter in brains of patients with multiple sclerosis (MS) (*2–4*). In studies of the pathogenesis of MS, T lymphocytes have been identified in the demyelinated regions of white matter as well as in clusters in apparently normal white matter far from infiltrating blood vessels (*5, 6*). Since factors stimulating mixed glial cultures have been shown to be produced by peripheral blood B cells or T cells in the presence of macrophages (*7*), we hypothesized that T cells participating in inflammatory reactions in the brains of MS patients could produce factors that induce proliferation and maturation of astrocytes and oligodendrocytes. We report that astrocytes and oligodendroglial cells proliferate in response to factors produced by the human T lymphocyte line Mo (*8*), human T cell leukemia-lymphoma virus (HTLV)–transformed peripheral blood T cells from healthy individuals (*9–11*), and phytohemagglutinin (PHA)-activated T cells from peripheral blood and cerebrospinal fluid of MS patients and normal control individuals. Factors produced by Mo cells also induce changes in surface and cytoplasmic markers of glial cells, some of which indicate accelerated maturation of these cells.

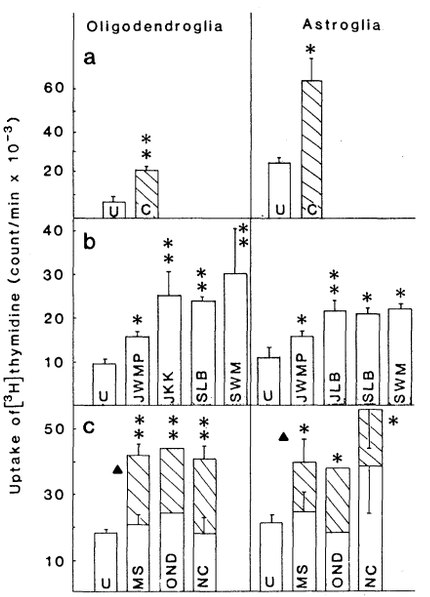

Fig. 1. Effect of activated T cell conditioned media on glial cell proliferation. Oligodendroglial cells were separated from adherent astrocytes by mechanical agitation in 1-week-old cultures established from 3-day-old rat brain. After treatment with antibody and complement to delete contaminating cells, glial cells were reseeded in Falcon Microtest II plates for 4 days in the presence or absence of putative growth-promoting supernatants (final concentration, 25 percent). Conditioned (*C*) and unconditioned (*U*) supernatants contained 2 percent PHA (final concentration in the Falcon plates, 0.05 percent). All cell line supernatants were generated identically: the cell line in serum-free Iscove's medium plus 2 percent PHA was incubated for up to 7 days at 37°C, harvested, and sterile-filtered. JLB, JWMP, and JKK are HTLV-II–containing transformants generated by cocultivation with Mo cells (*9, 14, 17*). SLB and SWM are HTLV-I–positive transformants generated by cocultivation with ME cells (*9, 14*). Supernatants from T cells of normal healthy controls (*NC*; $n = 13$), one other neurological disease (*OND*) patient, and multiple sclerosis (*MS*; $n = 18$) patients' peripheral blood and cerebrospinal fluid ($n = 2$) were generated by incubating the nylon wool–passed cells with or without 1 percent PHA for 5 days in serum-free medium containing 0.25 percent bovine serum albumin. Similar results were obtained with E-rosette–enriched T cells. Open bars in (c) represent cells in media without PHA; total height of bars (open plus filled) represents cells plus PHA; filled triangles represent cerebral spinal fluid cells plus PHA. Glial cells were exposed to [^{3}H]thymidine (1 μCi per well) and harvested after 17 hours with an automated harvester. Data in (a) and (c) are means ± standard errors for triplicate samples from ten representative experiments. Data in (b) are means ± standard deviations for triplicate samples from one experiment. Statistical significance was evaluated with a two-tailed paired Student's *t*-test comparing conditioned media with control (unconditioned) medium [(*) $P < 0.01$ and (**) $P < 0.001$]. Supernatants from peripheral blood and cerebrospinal fluid cells without PHA did not significantly differ from unconditioned medium (c). In parallel, cells were recovered by trypsinization from cultures with or without Mo cell supernatants and the number of viable cells was determined by trypan blue exclusion. Quadruplicate samples were counted. Oligodendrocyte cell counts gave $1.06 \pm 0.11 \times 10^4$ cells per well in unconditioned medium and $3.99 \pm 0.75 \times 10^4$ cells per well in Mo-conditioned medium ($P < 0.04$). After 4 days in unconditioned and Mo-conditioned media there were $1.93 \pm 0.29 \times 10^3$ and $5.00 \pm 0.63 \times 10^3$ astrocytes per well, respectively ($P < 0.05$).

Conditioned media were generated by incubating cell lines (Mo and HTLV-I and -II transformants) (*9–11*) for 4 to 7 days in Iscove's medium with or without 2 percent PHA (Wellcome). Unconditioned and control cell–conditioned media were also generated. Blood lymphocytes from healthy volunteers and MS patients were exposed to 1 percent PHA for 5 days, at which time supernatants were harvested.

Primary glial cell cultures were established from neonatal rat cerebrums by the method of McCarthy and DeVellis (*12*). After 7 days in primary culture, oligodendrocytes were separated from astrocytes and established in culture with or without growth factor–containing supernatants. Oligodendroglial cells were first treated with antibody to rat Thy 1 (clone OX7HL, 1:100; Sera-Lab) and antibody to fibronectin (1:20; Accurate) plus guinea pig complement (C′) (1:10) for 30 minutes at 37°C twice to eliminate contaminating astrocytes and neurons positive for Thy 1 and fibroblasts positive for Thy 1 and fibronectin. Astrocytes were first treated with antibody to fibronectin plus C′ to eliminate fibroblasts, antibody to Thy 1 for neurons, and antibody to galactocerebroside (rabbit antibody generated in our laboratory; 1:1000) plus C′ to eliminate contaminating oligodendrocytes. Oligodendroglia and astrocytes were then seeded at a density of 10^4 and 10^3 cells per well, respectively.

Supernatants from Mo cell–conditioned media were found to increase proliferation of glial cells, as determined by uptake of [^{3}H]thymidine, total cell counts (Fig. 1a), and autoradiography of cells positive for galactocerebroside (Fig. 2). Glial proliferation began by day 2, peaked on day 4, and decreased by day 6. Thus a harvest obtained on day 4 was used. Glial proliferation increased in a linear fashion when 5 to 50 percent final concentrations of supernatants were used. At concentrations above 50 percent, the supernatants had no further enhancing effect, and in some cases were inhibitory. A 25 percent final concentration was therefore used. These glial growth-promoting factors were produced constitutively like some other Mo lymphokines (*13, 14*), but their production could in some cases be augmented by PHA. Preliminary results suggest that the Mo growth factor has a molecular weight of 6,000 to 10,000, thus differentiating it from glial stimulating factor (*15*).

Glial growth-promoting lymphokines are the products of activated T lymphocytes (Fig. 1). The Mo line, derived from

a patient with hairy cell leukemia, is composed of neoplastic T cells infected with HTLV-II (*16, 17*). Lymphokine-producing transformants have been derived by cocultivation of peripheral blood T lymphocytes from healthy adults with lethally irradiated Mo cells (*9, 11, 17*). HTLV-II transformant–conditioned media (JLB, JWMP, and JKK) significantly increased glial proliferation (Fig. 1b). SLB and SWM, which also produce glial proliferation factors, are HTLV-I–infected T cell lines established by cocultivation of normal T lymphocytes with ME (*9, 10*), an HTLV-I–infected human T cell leukemia cell line (Fig. 1b). While resting peripheral blood T cells did not elaborate growth-promoting factors (Fig. 1c), PHA-induced blood and cerebrospinal fluid T cells produced significant levels of such factors (Fig. 1c). Supernatants from the T cell line HSB2, containing interleukin-2 (*18*), did not stimulate proliferation of astrocytes or oligodendrocytes, nor did purified interleukin-2 at final concentrations of 50, 25, and 10 percent. Products of the T lymphoblastoid cell line CEM, the erythroid line K562, and the myeloid line HL60 did not significantly stimulate either type of glial cell to proliferate more than controls, illustrating that non–T cells and immature T cells that do not elaborate lymphokines do not produce glial growth-promoting factors. T cell "activation" is not restricted to one type of virus nor is it virus-dependent, since PHA-stimulated lymphocytes also induce these factors. Presumably the MS antigens induce these factors in vivo as well. Primary fibroblasts were not induced to proliferate in response to Mo cell supernatants (from two different passages shown to give stimulation of glial cells) or to PHA-induced factors of normal T lymphocytes or T lymphocytes purified from MS patients.

In addition to producing factors inducing proliferation (*13, 14*), the Mo line also produces factors affecting differentiated functions of a variety of cell types (*19, 20*). To study the enhanced maturation of glial cells after exposure to Mo cell–conditioned medium and to exclude the possibility that contaminating cell types grew out instead of the glial cells seeded, we analyzed glial cell surface and cytoplasmic markers after 4 days in culture in the absence or presence of Mo cell–conditioned medium. All primary antibodies were centrifuged at 15,000*g* for 1 hour before use to avoid nonspecific staining due to absorption of antibodies by Fc receptors on oligodendroglia (*21*) when staining the surface of living cells. Secondary rabbit or mouse antibodies conjugated to fluorescein isothiocyanate (FITC) were $F(ab')_2$ fragments; these were also centrifuged before use. Normal rabbit and mouse serum controls gave no reaction, indicating that when immunoglobulin G (IgG) aggregates are removed by high-speed centrifugation before use, primary rat oligodendrocytes do not bind 7*S* IgG at Fc receptors.

Table 1. Effect of Mo-conditioned medium on cytoplasmic and surface markers of primary glial cells. Oligodendroglial cells were prepared as described in the legend to Fig. 1 and incubated in Costar 24-well cluster plates on sterile cover slips for 4 days with or without Mo supernatants. At the end of this period, cells were stained for surface markers by using indirect immunofluorescence. Surface staining was performed for 30 minutes at room temperature with rabbit antibody to galactocerebroside (GalC) (1:1000), rabbit antibody to fibronectin (1:20; Accurate) followed by FITC-conjugated $F(ab')_2$ fragments of goat antibody to rabbit Ig (1:20; Accurate), mouse monoclonal antibodies to rat Thy 1 (1:100; Sera-Lab), to rat Ia's (clone OX6HL, 1:1000; Sera-Lab), to rat T cells (clone WS/13 HLK, 1:100; Sera-Lab), to TAC (1:500) followed by FITC-conjugated $F(ab')_2$ fragments of goat antibody to mouse Ig (1:20; Accurate). FITC-conjugated lectins (Sigma) were used to visualize lectin receptors. Surface tetanus toxin receptors were stained with tetanus toxin, human antiserum to tetanus toxin, and FITC-conjugated rabbit antibody to human Ig (Accurate). Cytoplasmic staining was performed on fixed cells with rabbit antibody to glial fibrillary acidic protein (GFAP) (1:10), antibody to glycerol-3-phosphate dehydrogenase (G3PDH), FITC-conjugated goat antibody to rabbit Ig (1:20) or mouse monoclonal antibody to myelin basic protein (MBP) (1:100), and FITC-conjugated fragments of goat $F(ab')_2$ antibodies to mouse Ig (1:20). Symbols: +, 10 to 15 percent of the cells scored were positive for the marker indicated; ++, 50 to 60 percent; and +++, 100 percent. Additional abbreviations: Con A, concanavalin A; PWM, pokeweed mitogen; SBA, soybean agglutinin; WGA, wheat germ agglutinin; and PNA, peanut agglutinin.

Marker	Unconditioned medium	Mo-conditioned medium
GFAP	–	–
GalC	+++	+++
MBP*	±	+
G3PDH*	–	++
Fibronectin	–	–
Rat Thy 1	–	–
TAC*	±	++
Tetanus toxin	–	–
Con A receptor	++	+++
PHA	±	±
PWM*	+	++
SBA*	–	+++
WGA*	++	+++
Lentil	++	+++
PNA*	+	++

*Cell makers that changed significantly after exposure to Mo-conditioned medium.

Oligodendrocyte cultures were negative for glial fibrillary acidic protein (Dako PAPkit, Immulok); that is, astrocytes did not overgrow the oligodendrocytes and were not responsible for uptake of [^{3}H]thymidine (Table 1). Of 1000 cells in the oligodendrocyte-enriched population stained with antibody to glial fibrillary acidic protein and counterstained with hematoxylin, only 2.05 ± 0.85 percent were astrocytes. The cultures were determined to be 96.7 ± 2.3 percent oligodendrocytes by counting 1200 cells stained with antibody to galactocerebroside and counterstained with hematoxylin (*22, 23*). The combination of (i) staining with peroxidase-antiperoxidase (PAP) and antibody to galactocerebroside and (ii) autoradiography proved that oligodendrocytes were the most common type of cell proliferating in the oligodendrocyte-enriched cultures (Fig. 2); 68 percent of these cells were dividing in cultures exposed to Mo cell supernatants. There were no contaminating fibroblasts [cultures were negative for fibronectin and Thy 1) or neurons (negative for Thy 1 and tetanus toxin receptor (*22*)] in the oligodendrocytes (Table 1). Under the influence of Mo-conditioned medium for 4 days, oligodendroglia expressed markers characteristic of mature oligodendrocytes not normally seen for a much longer period in culture or only after induction. Myelin basic protein, usually seen only at 21 days (*24*), was seen by day 11. Glycerol-3-phosphate dehydrogenase (*25*) was induced by Mo supernatants, although previously it had been shown to be induced only by corticosteroids (*26*). Mo cell–conditioned medium did not induce surface I region–associated antigens (Ia's) (*27*) or T cell surface markers (*28*) on oligodendroglia, and, while some cells did develop TAC antigens (interleukin-2 receptors) (*29*), they did not respond to interleukin-2. Concanavalin A binding sites, previously identified on oligodendroglia (*30*), and binding sites for wheat germ agglutinin and soybean agglutinin associated with myelin (*31*) were also identified on the surfaces of oligodendroglia. Expression of receptors for pokeweed mitogen, soybean agglutinin, and peanut agglutinin, an event that usually does not occur until day 21 in primary cultures, occurred after a 4-day exposure to Mo factors. These accelerated changes in differentiation of oligodendrocytes are additional effects of factors secreted by the Mo line.

Astrocytes showed fewer changes in differentiation markers. None were Thy 1–positive, perhaps because of their cerebral cortex origin (*32*), nor did they

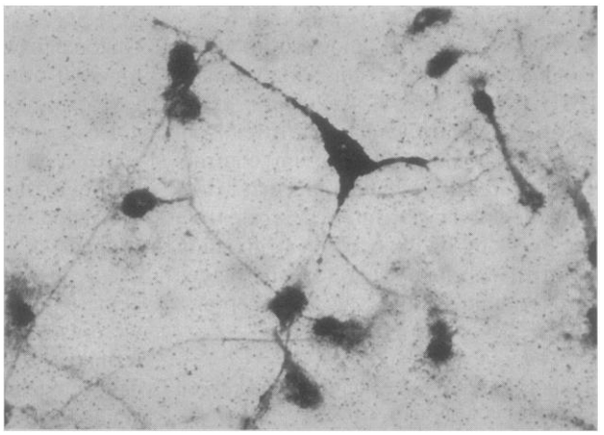

Fig. 2. Oligodendroglial cells proliferating in response to Mo-conditioned medium. Oligodendroglial cells prepared as described in text were grown for 4 days in Mo-conditioned medium on sterile cover slips in Costar 24-well plates. They were labeled with [^{3}H]thymidine for 17 hours before harvest. At harvest the cells were stained with rabbit antibody to galactocerebroside (1:20) followed by swine antibody to rabbit Ig (1:200; Dako). This was followed in turn by staining with rabbit PAP (1:200; Dako). All antibodies were centrifuged (10,000*g* for 1 hour) to remove complexes. Aminoethylcarbazol was the substrate used. Stained slides were immersed in Ilford K2 nuclear emulsion, exposed for 2 days at 4°C, and developed.

develop T cell antigens or Ia's. There were no contaminating oligodendrocytes, neurons, or fibroblasts in astrocyte cultures. Astrocytes expressed different lectin receptors than oligodendrocytes. While asparagus pea and pokeweed mitogen lectin receptors usually are not seen in primary astrocyte cultures as old as 1 month, they developed under the influence of medium conditioned with T cells. The appearance of these receptors means that the lectin-binding sugar or a glycoprotein containing the sugar develops de novo under the influence of Mo factors.

Recently, Raff *et al.* (*33*) demonstrated a bipotential glial progenitor cell in rat optic nerve bearing the A2B5 antigen. This cell can differentiate into an oligodendrocyte or an astrocyte. If we treated the astrocyte- and oligodendrocyte-enriched populations from 7-day-old cultures with antibody to A2B5 (1:100; Sera-Lab) and C′ to remove such precursors before incubation with Mo supernatants, we were still able to demonstrate glial proliferation by [^{3}H]thymidine uptake, cell counts, and autoradiography. This indicates that both distinct glial cell types and not precursor cells constituted the dividing populations (Fig. 2). These results, like those of Raff *et al.* (*33*), indicate that glial cells of the central nervous system are highly plastic and are able to respond to hormones or lymphokines present during development or in an inflammatory environment by undergoing expansion and differentiation.

Hyperplasia of glial cells in the brain is usually a response to injury (*1–3*). In MS, astrocyte hyperplasia and gliosis result in brain plaques that cause some of the clinical symptoms. Oligodendroglial cells also undergo some hyperplasia (*1*). In normal white matter adjacent to the plaques increased numbers of oligodendrocytes suggest proliferation in association with remyelination (*4*). Subsequently they are damaged and disappear (*2–4*). Proliferation of oligodendroglial cells thus appears to be a potential mechanism of repair and remyelination, as it is in animal models of demyelination involving JHM virus and recurrent experimental allergic encephalomyelitis (*34*). Normal brains contain no leukocytes. Thus T cells may contribute to the disease process in MS, since activated T cells are part of the inflammatory infiltrate (*35, 36*) and since some are even seen migrating deep into normal-appearing white matter (*5, 6*). It would be desirable to be able to produce oligodendroglial proliferation factors and suppress astroglial factors. Our results show that cerebrospinal fluid and peripheral blood T cells, when stimulated by PHA, make factors as stimulatory as those produced by the Mo line and HTLV-I and -II transformants. Characterization of factors produced by activated T cells may help to elucidate the pathogenesis of glial hyperplasia in MS as well as in other diseases involving chronic inflammation in the presence of infiltrating lymphocytes.

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References and Notes

1. S. K. Ludwin, *Nature (London)* **308**, 274 (1984).
2. C. W. M. Adams, *Br. Med. J.* **33**, 15 (1977).
3. J. Prineas, *Hum. Pathol.* **6**, 531 (1975).
4. C. S. Raine, L. Scheinberg, J. M. Waltz, *Lab. Invest.* **45**, 534 (1981).
5. H. W. Kreth, R. Dunker, H. Rodt, R. Meyermann, *J. Neuroimmunol.* **2**, 177 (1982).
6. U. Traugott, E. L. Reinherz, C. S. Raine, *ibid.* **3**, 365 (1982).
7. A. Fontana, R. Dubs, R. Merchant, S. Balsiger, P. J. Grub, *J. Neuroimmunol.* **2**, 55 (1981).
8. A. Saxon, R. H. Stevens, D. W. Golde, *Ann. Intern. Med.* **88**, 323 (1978).
9. J. C. Gasson, I. S. J. Chen, C. A. Westbrook, D. W. Golde, in *Normal and Neoplastic Hematopoiesis*, vol. 9 of UCLA Symposia on Molecular and Cellular Biology, New Series, D. W. Golde and P. A. Marks, Eds. (Liss, New York, 1983), p. 129.
10. H. P. Koeffler, I. S. Y. Chen, D. W. Golde, *Blood*, in press.
11. I. S. Y. Chen, S. G. Quan, D. W. Golde, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7006 (1983).
12. K. D. McCarthy and J. J. DeVellis, *Cell Biol.* **85**, 890 (1980).
13. A. J. Lusis, D. H. Quan, D. W. Golde, *Blood* **57**, 13 (1981).
14. D. W. Golde, N. Bersch, S. G. Quan, A. J. Lusis, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 593 (1980).
15. A. Fontana, U. Otz, A. L. deWeck, P. J. Grob, *J. Neuroimmunol.* **2**, 73 (1981).
16. V. S. Kalyanaraman, M. G. Sarngadharan, M. Robert-Guroff, I. Miyoshi, D. Blayney, D. Golde, R. C. Gallo, *Science* **218**, 571 (1982).
17. I. S. Y. Chen, J. McLaughlin, J. C. Gasson, S. C. Clark, D. W. Golde, *Nature (London)* **305**, 502 (1983).
18. D. M. Stadler and J. J. Oppenheim, *Lymphokines* **6**, 117 (1982).
19. I. Nathan, J. E. Groopman, S. G. Quan, N. Bersch, D. W. Golde, *Nature (London)* **292**, 831 (1981).
20. R. H. Weisbart, A. J. Lusis, G. Chan, R. Billings, R. F. Ashman, D. W. Golde, *J. Immunol.* **128**, 457 (1982).
21. B. I. Ma, B. S. Joseph, M. J. Walsh, A. Potvin, W. W. Tourtellotte, *Ann. Neurol.* **9**, 371 (1981).
22. R. Mirsky, in *Neuroimmunology*, J. Brockes, Ed. (Plenum, New York, 1982), p. 141.
23. B. Ranscht, P. A. Clapshaw, J. Price, M. Noble, W. Seifer, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2709 (1982).
24. G. Roussel, G. Labourdette, J. L. Nussbaum, *Dev. Biol.* **81**, 372 (1981).
25. S. Varon, in *Dynamic Properties of Glial Cells*, E. Schoffeniels, G. Franck, L. Hertz, D. B. Tower, Eds. (Pergamon, New York, 1977), p. 93.
26. P. J. LeVeille, J. F. Mcginnis, D. S. Maxwell, J. DeVellis, *Brain Res.* **196**, 287 (1980).
27. J. P. T. Ting, B. L. Shigekawa, D. S. Linthicom, L. P. Weiner, J. A. Frelinger, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3170 (1981).
28. J. S. Oger, J. Szuchet, D. G. W. Antel, I. Arnason, *Nature (London)* **295**, 66 (1982).
29. W. J. Leonard, J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldman, W. C. Greene, *ibid.* **300**, 267 (1982).
30. L. M. Garcia-Segura, R. Martinez-Rodriquez, R. Martinez-Murillo, I. Suarez, *Gegenbaurs Morphol. Jahrb.* **5**, 637 (1978).
31. J. F. Poduslo, J. L. Harman, D. E. McFarlin, *J. Neurochem.* **34**, 1733 (1980).
32. R. Pruss, *Nature (London)* **280**, 688 (1979).
33. M. C. Raff, R. H. Miller, M. Nobel, *ibid.* **303**, 390 (1983).
34. P. W. Lampert, *Am. J. Pathol.* **91**, 176 (1978).
35. M. I. Reunanen, *J. Neuroimmunol.* **3**, 275 (1982).
36. A. B. C. Noronha, D. P. Richman, D. G. W. Arnason, *N. Engl. J. Med.* **303**, 713 (1980).
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