Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Regulation of astrocyte proliferation by prostaglandin E₂ and the α subtype of protein kinase C

Makoto Sawada a, Akio Suzumura b, Kazushige Ohno a,* and Tohru Marunouchi a

a Division of Cell Biology, Institute for Comprehensive Medical Science and b Department of Neurology, School of Medicine, Fujita Health University, Aichi (Japan)

(Received 29 December 1992)

Key words: Astrocyte growth regulation; Prostaglandin; α Subtype of protein kinase C; 12-O-Tetradecanoylphorbol 13-acetate; Brain cell culture

We found that astrocytes expressed the α subtype of protein kinase C. Treatment with 12-O-tetradecanoylphorbol 13-acetate (TPA) caused cultured astrocytes to proliferate. This effect of TPA was blocked by staurosporine, a potent protein kinase C inhibitor, suggesting the involvement of protein kinase C in astrocyte proliferation. Indomethacin, an inhibitor of prostaglandin formation, enhanced both the normal and TPA-induced proliferation of astrocytes. Authentic prostaglandin E₂ blocked this effect of indomethacin and also partially blocked the effect of TPA, suggesting that the intracellular mechanisms involved in prostaglandin E₂-regulated astrocyte growth might differ from those acting in protein kinase-dependent growth. The effect of prostaglandin E₂ was blocked by a specific anti-prostaglandin E₂ polyclonal antibody. Cultured astrocytes and microglia produced and released prostaglandin E₂ in response to stimulants such as lipopolysaccharide, TPA, and lymphokines. Since the sensitivity of astrocytes and microglia to these stimuli was different, prostaglandin E₂ may differentially regulate astrocyte proliferation under different physiological conditions, acting in an autocrine fashion for astrocytes and in a paracrine fashion for microglia.

INTRODUCTION

Astrocytes are a type of glial cell and they provide structural support for neurons. There is also growing evidence that astrocytes have additional functions since they have been shown to synthesize and/or respond to a variety of growth factors and cytokines, including interleukin 1, interleukin 6, granulocyte-macrophage colony stimulating factor, and tumor necrosis factor α. It has also been demonstrated that certain factors control both the activity and proliferation of astrocytes via intracellular signaling mechanisms. Protein kinase C is one of the key enzymes involved in intracellular signaling and its activation has been implicated in a wide range of cellular processes. One of the most important roles of protein kinase C is modulation of the process of cell growth and division. Brain tissue has the highest protein kinase C content in the body, and the density and distribution of phorbol ester binding sites in the fetal brain suggest a role for this enzyme in both developmental processes and cell growth. The existence of protein kinase C has also been demonstrated in primary astrocyte cultures and it has been shown that mediators such as neurotransmitters or growth factors can stimulate protein kinase C activity. A phorbol ester was recently shown to induce the proliferation of cultured astrocytes. Therefore, protein kinase C seems to be involved in the promotion of astrocyte growth both in the developmental stage and under certain pathological conditions.

Molecular cloning of the cDNA for protein kinase C has recently clarified the existence of multiple subtypes of this enzyme, and there appear to be at least seven subtypes (α-ζ, including two β subseries) of the mammalian brain has been shown to contain at least four subtypes, α, βI, βII, and γ, by immunohistochemical analysis; they were localized in neurons and in glial cells. Like the cells of the immune system, brain cells such...
Fig. 1. A: staining of cultured astrocytes for GFAP. B: staining of cultured astrocytes for the α subtype of protein kinase C. Bars in A and B = 15 and 30 μm, respectively.
as astrocytes and microglia have also been shown to produce prostaglandin E₂, although its function in the central nervous system is as yet unknown. An attractive hypothesis is that growth factors or cytokines may activate astrocyte proliferation and subsequently induce prostaglandin E₂ production, which then down-regulates astrocyte growth in an autoregulatory circuit. This hypothesis has been supported by a number of studies on lymphocytes, which have demonstrated that prostaglandin E₂ suppresses the activity or growth of both macrophages and T cells. However, the effects of prostaglandin E₂ on astrocyte proliferation have not yet been investigated directly.

In this study, we showed that a phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) could induce astrocyte proliferation, most likely via activation of the α subtype of protein kinase C, and that prostaglandin E₂ inhibited both the normal and TPA-stimulated proliferation of cultured astrocytes.

MATERIALS AND METHODS

Materials

Bovine insulin, indomethacin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and lipopolysaccharide (LPS) were obtained from Sigma (St. Louis, MO, USA). Prostaglandin E₂ and anti-prostaglandin E₂ antibody were obtained from Funakoshi (Tokyo, Japan). Staurosporine was purchased from Kyowa Medex (Tokyo, Japan). All other reagents used were of the highest purity commercially available. A crude lymphokine preparation was obtained by the method of Suzumura et al. Preparations of astrocytes and microglia

Astrocytes were prepared from primary mixed glial cell cultures of normal newborn ICR mouse and were purified by 3–4 cycles of trypsinization and replating, as described previously. The purity of the astrocytes thus obtained was more than 95% as determined by indirect immunofluorescence using an anti-glial fibrillary acidic protein antibody. These astrocyte-enriched cultures did not contain neurofilament-positive neurons and the contamination of microglia was negligible. Microglia were prepared as described previously. The purity of microglia was more than 98% as determined by indirect immunofluorescence using an anti-Mac1 antibody.

Immunostaining of protein kinase C subtypes

Purified astrocytes were plated on 14 mm diameter glass coverslips at a density of 2.5 × 10⁴ cells/ml. After 3 days of culture, they were immunolabeled with isozyme-specific anti-protein kinase C antibodies (directed against the α, β and γ subtypes), using a commercially available protein kinase C staining kit (MBL, Nagoya, Japan).

Treatment of astrocytes

Isolated astrocytes were seeded at a density of 2.5 × 10⁴ cells per well in 24-well Falcon testplates. After 12 h of culture, they were stimulated with TPA, LPS, or a crude lymphokine preparation. For the inhibition test, a potent protein kinase inhibitor, staurosporine, was added to the medium in the presence of 100 ng/ml TPA. In addition, various concentrations of indomethacin (an inhibitor of prostaglandin production) were added to the medium in the presence or absence of TPA or indomethacin. Cultures were maintained in Eagle’s MEM supplemented with 3.5 g/l glucose and 5 mg/l bovine insulin for 3 or 5 days at 37°C, and then assayed.

Measurement of cell proliferation

The proliferative activity of astrocytes was determined by a modification of the MTT colorimetric method. MTT is cleaved by living cells to yield a formazan product and this process requires active mitochondria. Thus, measurement of the formazan product by a colorimetric assay gives an indication of the number of surviving cells. Cells in 24-well test plates were washed twice with phosphate-buffered saline (pH 7.2) and treated with 0.5 ml of a culture medium containing 0.5 mM MTT. After incubation at 37°C for 6 h, 0.04 M HCl-isopropanol was added, the amount of the formazan product in 0.2 ml of the culture fluid was measured at OD 620 nm with a J2000 Immuno Reader (Inter Med Japan Co., Tokyo, Japan).

Measurement of prostaglandin E₂

Prostaglandin E₂ concentrations in the medium from cultured astrocytes and microglia were measured by radioimmunoassay with an anti-prostaglandin E₂ antibody (Amersham Japan, Tokyo, Japan).

Statistics

Control and experimental values were compared using Student’s t-test.

RESULTS

The purity of the cultured astrocytes was determined to be more than 95% by GFAP immunostaining with the anti-GFAP antibody (Fig. 1A). These astrocytes contained the α subtype of protein kinase C (Fig. 1B), but β and γ subtypes were not detected in our experiment (data not shown).

The amount of formazan product detected at 620 nm showed a good correlation to astrocyte numbers (Fig. 2), so the colorimetric assay was validated for assessing astrocyte proliferation in the subsequent experiments.

When astrocyte cultures were treated with three stimulants (TPA, LPS and crude cytokine extract) that have been shown to activate these cells in different manners, only TPA was found to enhance astrocyte proliferation (Fig. 3A). TPA enhanced astrocyte proliferation in a dose-dependent manner from a concentration of 0.1 to 100 ng/ml, but was toxic at higher concentrations (Fig. 3B). Since the cultures were not confluent, the astrocytes gradually increased in numbers, showing a 25% increase on day 3 and a 45% increase on day 5 (Fig. 3C). This time-dependent increase was enhanced by TPA to about 40% on day 3 and 55% on day 5, when compared to the respective control (Fig. 3C). The effect of TPA was inhibited by 10 nM staurosporine (Fig. 3C).

Indomethacin (1 μM) increased both the control and TPA-induced proliferation of astrocytes by 260% and 370%, respectively (Fig. 4). This effect of indomethacin showed saturation at around 10 μM. It
also increased astrocyte proliferation in the presence of 10 nM staurosporine, which completely inhibited TPA-dependent growth; 10 μM indomethacin increased astrocyte proliferation slightly but significantly (Fig. 4).

Authentic prostaglandin E₂ inhibited both the indomethacin-induced and control proliferation of astrocytes (Fig. 5), and it reduced the TPA-induced proliferative response of astrocytes to only 30% (Fig. 5). These effects of prostaglandin E₂ were reversed by addition of the anti-prostaglandin E₂ antibody (Fig. 5). Treatment with this antibody increased astrocyte prolifera-

---

**Fig. 2.** The relationship between astrocyte numbers and formazan production from MTT. The indicated number of astrocytes were seeded in a 24-well testplate, cultured for 5 days and subjected to the MTT assay as described in Materials and Methods. The amount of formazan produced was measured at 620 nm. Each value indicates the mean of quadruplicate samples from two different experiments.

**Fig. 3.** A: effects of various stimulants on astrocyte proliferation. Astrocytes \(2.5 \times 10^4\) were incubated with 100 ng/ml of TPA, 1 μg/ml LPS, 10% (v/v) crude cytokine extract, or medium alone (control) for 5 days. Columns and bars represent the mean ± S.D. from 5 different experiments and shown the percentage difference from untreated control cultures (which were taken as 100%). The control value was 0.122 ± 0.012. **P < 0.01. B: dose-dependent increase of astrocyte proliferation in response to TPA. Astrocytes \(2.5 \times 10^4\) were incubated with the indicated concentrations of TPA for 5 days. Points and bars represent the mean ± S.D. of 3 different experiments and show the percentage of the untreated control proliferation. The control value was 0.135 ± 0.010. *P < 0.05. **P < 0.01. C: time course of astrocyte proliferation. Astrocytes \(2.5 \times 10^4\) were incubated with 100 ng/ml TPA in the presence (▲) or absence (●) of 10 nM staurosporine or medium alone (×) for the indicated number of days. Points and bars represent the mean ± S.D. of 5 different experiments and show the percentage of the value on day 0 of culture. The control value was 0.098 ± 0.011. *P < 0.05. **P < 0.01.

**Fig. 4.** Effects of indomethacin on astrocyte proliferation. Astrocytes \(2.5 \times 10^4\) were incubated for 5 days with the indicated concentrations of indomethacin and (△) 100 ng/ml TPA, (●) 100 ng/ml TPA + 10 nM staurosporine, or (×) medium only. Points and bars represent the mean ± S.D. of 5 different experiments and show the percentage of the value on day of treatment. The control value is 0.098 ± 0.011. *P < 0.05. **P < 0.01.
"Fig. 5. Effects of prostaglandin E\textsubscript{2} on astrocyte proliferation. Astrocytes (2.5 \times 10^4) were incubated with prostaglandin E\textsubscript{2} (5 mg/ml was added once daily for 5 days) in the presence (hatched columns) or absence (filled columns) of an anti-prostaglandin E\textsubscript{2} antibody (1:500 dilution) or medium only (open columns). Similar experiments were performed in the presence of 1 \mu M indomethacin or 100 ng/ml TPA. Columns and bars represent the mean\pm S.D. of 6 different experiments and show the percentage of the untreated control value. The control value was 0.125\pm 0.008. * P < 0.05 when compared to the respective control. * P < 0.05 when compared as indicated.

Astrocytes produced about 200 pg of prostaglandin E\textsubscript{2} per 2 \times 10^5 cells under control culture conditions, an amount that was four times the production by microglia under the same conditions (Fig. 6). LPS and TPA increased prostaglandin E\textsubscript{2} production about 1.7-fold and 4.5-fold, respectively, when compared to the control astrocyte cultures. LPS and TPA also increased microglial prostaglandin E\textsubscript{2} production by about 40-fold and 5-fold, respectively. Thus, astrocytes were more sensitive to TPA than to LPS, while microglia were the opposite.

Fig. 6. Production of prostaglandin E\textsubscript{2} by astrocytes and microglia. Astrocytes (5 \times 10^5, hatched columns) and microglia (5 \times 10^5, filled columns) were incubated with or without LPS (0.1 \mu g/ml), TPA (0.1 \mu g/ml), or crude lymphokines (1%) for 24 h. Supernatant fractions were then collected and the prostaglandin E\textsubscript{2} content was measured with a radioimmunoassay kit. The values indicate the mean of two independent experiments.

DISCUSSION

There are two major aspects to consider with regard to astrocyte proliferation, one being its contribution to normal brain ontogeny\textsuperscript{17} and the other being its pathological role in causing gliosis in the mature brain\textsuperscript{17,22}. In the latter situation, quiescent astrocytes re-enter the cell cycle like somatic cells such as skin fibroblasts, gut epithelial cells, and hepatocytes\textsuperscript{34,35}. Despite the physiological and pathological importance of this cell, relatively little is known about the intracellular and intercellular mechanisms regulating astrocyte proliferation.

We demonstrate here that TPA stimulated the proliferation of cultured astrocytes, and that this effect was blocked by staurosporine, a potent protein kinase C inhibitor\textsuperscript{48} (Fig. 2). Staurosporine also inhibited unstimulated astrocyte proliferation (Fig. 2). These observations suggest that protein kinase C may be involved in astrocyte proliferation under both unstimulated and TPA-stimulated conditions. This hypothesis was supported by the result that astrocytes expressed protein kinase C (Fig. 1B). Glial cells are reported to be rich in immunoreactive protein kinase C\textsuperscript{46} and a [\textsuperscript{3}H]phorbol ester has been found to bind to glial cells at a high level\textsuperscript{4}. In general, when cells are stimulated with TPA, protein kinase C activity is translocated from the cytosol to the membrane\textsuperscript{2} or to the nucleus\textsuperscript{27}. Translocation of its activity to the nucleus seems to be necessary for DNA synthesis, suggesting that protein kinase C may be a key enzyme involved in cell proliferation. A similar mechanism may be involved in the astrocyte proliferation.

Indomethacin, a prostaglandin synthesis inhibitor, enhanced both unstimulated and TPA-stimulated astrocyte proliferation (Fig. 4), suggesting that astrocyte-derived prostaglandins reduced its own proliferation in both unstimulated and TPA-stimulated conditions. To identify what type of prostaglandins inhibit astrocyte proliferation, we added the authentic prostaglandins to astrocyte cultures. An excess of prostaglandin E\textsubscript{2} reduced both unstimulated and stimulated proliferation of astrocytes (Fig. 5). This inhibitory effect was blocked by an antibody to prostaglandin E\textsubscript{2} (Fig. 5). These findings indicate that this prostaglandin apparently functions as an inhibitory regulator of astrocyte proliferation.

We also demonstrated that prostaglandin E\textsubscript{2} partially blocked the effect of TPA on astrocytes (Fig. 5), suggesting that different intracellular signaling mecha-
Acknowledgements. This work was supported in part by Grants-In-Aid for Scientific Research from Fujita Health University, Yamanouchi Pharmaceutical Company Ltd., Japanese Ministry of Education, Science and Culture, Japanese Ministry of Health and Welfare, and Human Science Foundation, and in part by the Science Research Promotion Fund from the Japan Private School Promotion Foundation.

Abbreviations

GFAP glial fibrillary acidic protein
LPS lipopolysaccharide
MIT 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
TPA 12-O-tetradecanoylphorbol 13-acetate

References

1. Berridge, M.J., Intracellular signaling through inositol tris-phosphate and diacylglycerol, *Biol. Chem. Hoppe-Seyler*, 367 (1986) 447–456.
2. Bhat, N.R., Role of protein kinase C in glial cell proliferation, *J. Neurosci. Res.*, 22 (1989) 20–27.
3. Bolton, C., Gordon, D. and Turk, J.L., A longitudinal study of the prostaglandin content of central nervous system tissues from guinea pigs with acute experimental allergic encephalomyelitis, *Int. J. Immunopharmacol.*, 6 (1984) 155–161.
4. Burch, R.M. and Kniss, D.A., Modulation of receptor-mediated signal transduction by diacylglycerol mimetics in astrocytes, *Cell. Moll. Neurobiol.*, 8 (1988) 251–257.
5. Coussens, L., Parker, P.J., Rhee, L., Yang-Feng, T.L., Chen, E., Waterfield, M.D., Francke, U. and Ullrich, A., Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways, *Science*, 233 (1986) 859–866.
6. Fierz, W., Endler, B., Reske, K., Wekerle, H. and Fontana, A., Astrocytes as antigen-presenting cells. I. Induction of la antigen expression on astrocytes by T cells via immune interferon and its effects on antigen presentation, *J. Immunol.*, 134 (1985) 3785–3793.
7. Frei, K., Malipiero, U.V., Leist, T.P., Zinkernagel, R.M., Schwab, M.E. and Fontana, A., On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases, *Eur. J. Immunol.*, 19 (1989) 689–694.
8. Fontana, A., Kristensen, F., Dubs, R., Gamsa, D. and Weber, E., Production of prostaglandin E and an interleukin-1 like factor by cultured astrocyte and C6 glioma cells, *J. Immunol.*, 129 (1982) 2413–2419.
9. Gamsa, D., Stimulation of prostaglandin E release from macrophages and possible roles in the immune response, *Lymphokines*, 4 (1981) 335–375.
10. Girard, P.R., Mazzci, G.J., Wood, J.G. and Kuo, J.F., Polyclonal antibodies to phospholipid/Ca2+-dependent protein kinase and immunocytocchemical localization of the enzyme in rat brain, *Proc. Natl. Acad. Sci. USA*, 82 (1985) 3030–3034.
11. Giulian, D., Baker, T.J., Shih, L.C.N. and Lachman, L.B., Interleukin-1 of the central nervous system is produced by ameboid microglia, *J. Exp. Med.*, 164 (1986) 594–604.
12. Goldyne, M.E. and Stobo, J.D., Immunoregulatory role of prostaglandins and related lipids, *CRC Crit. Rev. Immunol.*, 2 (1981) 189–223.
13. Goodwin, J.S. and Webb, D.R., Regulation of the immune response by prostaglandins, *Clin. Immunol. Immunopathol.*, 15 (1980) 106–122.
14. Hartung, H.-P. and Toyka, K.V., Phorbol diester TPA clicitis prostaglandin E release from cultured rat astrocytes, *Brain Res.*, 417 (1987) 347–349.
15. Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S. and Nishizuka, Y., Calcium-activated, phospholipid-dependent protein kinase from rat brain: subcellular distribution, purification and properties, *J. Biol. Chem.*, 257 (1982) 13341–13348.
16. Knopf, J.L., Lee, M.-H., Sultzman, L.A., Kriz, R.W., Loomis, C.R., Hewick, R.M. and Bell, R.M., Cloning and expression of multiple protein kinase C cDNAs, *Cell*, 46 (1986) 491–502.
17. Korr, H., Proliferation of different cell types in the brain. In A. Brodal, W. Hild, J. van Limbrough, R. Ortman, T.H. Schiebler, G. Tondury and E. Wolff (Eds.), *Advances in Anatomy, Embryology and Cell Biology, Springer-Verlag, New York*, 1980, pp. 5–16.
18. Makowske, M., Birnbaum, M.J., Ballester, R. and Rosen, O.M., A cDNA encoding protein kinase C identifies two species of mRNA in brain and GH3 cells, *J. Biol. Chem.*, 261 (1986) 13389–13392.
19. Malipiero, U.V., Frei, K. and Fontana, A., Production of hemopoietic colony-stimulating factors by astrocytes, *J. Immunol.*, 144 (1990) 3816–3821.
20 Merrill, J.E., Macroglia: neural cells responsive to lymphokines and growth factors, *Immunol. Today*, 6 (1987) 146–150.

21 Merrill, J.E., Gerner, R.H., Myers, L.W. and Ellison, G.W., Regulation of natural killer cell cytotoxicity by prostaglandin E in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis and other neurological diseases, *J. Neuroimmunol.*, 4 (1983) 223–237.

22 Miller, R.H., Abney, E.R., David, S., Ffrench-Constatn, C., Lindsay, R., Patel, R., Stone, J. and Raff, M.C., Is reactive gliosis a property of a distinct subpopulation of astrocytes? *J. Neurosci.*, 6 (1986) 22–29.

23 Minakuchi, R., Takai, Y., Yu, B. and Nishizuka, Y., Wide-spread occurrence of calcium-activated, phospholipid-dependent protein kinase in mammalian tissues, *J. Biochem.*, 89 (1981) 1651–1654.

24 Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, 65 (1983) 55–58.

25 Murphy, K.M.M., Gould, R.J., Oster-Granite, M.L., Gerhart, J.D. and Snyder, S.H., Phorbol ester receptors: autoradiographic identification in the developing rat, *Science*, 222 (1983) 1036–1038.

26 Neary, J.T., Norenberg, L.O.B. and Norenberg M.D., Calcium-activated, phospholipid-dependent protein kinase and protein substrates in primary culture of astrocytes, *Brain Res.*, 385 (1986) 420–424.

27 Nishizuka, Y., The role of protein kinase C in cell surface signal transduction and tumor promotion, *Nature*, 308 (1984) 693–698.

28 Nishizuka, Y., Studies and perspectives of protein kinase C, *Science*, 233 (1986) 1082.

29 Ohno, S., Kawasaki, H., Imajoh, S., Suzuki, K., Inagaki, M., Nishizuka, Y., Studies and perspectives of protein kinase C, *Nature*, 325 (1987) 354–358.

30 Ohno, K., Suzumura, A., Yamamoto, H. and Marunouchi, T., Macrophage-like properties of isolated mouse microglia cells, *Neurochem. Res.*, 13 (1988) 1082.

31 Ono, Y., Kikkawa, U., Nishizuka, Y. and Tanaka, C., Distribution of protein kinase C-like immunoreactive neurons in rat brain, *J. Neurosci.*, 8 (1988) 369–382.

32 Pardee, A.B., Dubrow, R., Hamlin, J.L. and Kletzein, R.R., Animal cell cycle, *Annu. Rev. Biochem.*, 47 (1978) 715–750.

33 Pardee, A.B., Coussens, L., Totty, N., Rhce, L., Young, S., Chen, E., Sabel, S., Waterfield, M.D. and Ullrich, A., The complete primary structure of protein kinase C - the major phorbol ester receptor, *Science*, 233 (1986) 853–859.

34 Parker, P.J., Coussens, L., Totty, N., Rhee, L., Young, S., Chen, E., Sabel, S., Waterfield, M.D. and Ullrich, A., The complete primary structure of protein kinase C - the major phorbol ester receptor, *Science*, 233 (1986) 853–859.

35 Pardee, A.B., Dubrow, R., Hamlin, J.L. and Kletzein, R.R., Animal cell cycle, *Annu. Rev. Biochem.*, 47 (1978) 715–750.

36 Parker, P.J., Coussens, L., Totty, N., Rhce, L., Young, S., Chen, E., Sabel, S., Waterfield, M.D. and Ullrich, A., The complete primary structure of protein kinase C - the major phorbol ester receptor, *Science*, 233 (1986) 853–859.

37 Pardee, A.B., Dubrow, R., Hamlin, J.L. and Kletzein, R.R., Animal cell cycle, *Annu. Rev. Biochem.*, 47 (1978) 715–750.

38 Parker, P.J., Coussens, L., Totty, N., Rhce, L., Young, S., Chen, E., Sabel, S., Waterfield, M.D. and Ullrich, A., The complete primary structure of protein kinase C - the major phorbol ester receptor, *Science*, 233 (1986) 853–859.

39 Sawada, M., Suzumura, A., Yamamoto, H. and Marunouchi, T., Macrophage-like properties of isolated mouse microglia cells, *Neurochem. Res.*, 13 (1988) 1082.

40 Sawada, M., Kondo, N., Suzumura, A. and Marunouchi, T., Production of tumor necrosis factor-alpha by microglia and astrocytes in culture, *Brain Res.*, 491 (1989) 394–397.

41 Sawada, M., Ohno, K., Kondo, N., Suzumura, A. and Marunouchi, T., Immunoregulatory roles of isolated microglia and astrocytes in culture, *Neurosci. Res.*, 9 (suppl.) (1989) s121.

42 Sawada, M., Suzumura, A., Yamamoto, H. and Marunouchi, T., Activation and proliferation of the isolated microglia by colony stimulating factor-1 and possible involvement of protein kinase C, *Brain Res.*, 509 (1990) 119–124.

43 Sawada, M., Suzumura, A. and Marunouchi, T., TNFα induces IL-6 production by astrocytes but not by microglia, *Brain Res.*, 583 (1992) 296–299.

44 Suzumura, A., Bhat, S., Eccleston, P.A., Lisak, R.P. and Silberberg, D.H., The isolation and long-term culture of oligodendrocytes from newborn mouse brain, *Brain Res.*, 324 (1984) 379–383.

45 Suzumura, A., Lavi, E., Weiss, S.R. and Silberberg, D.H., Induction of glial cell MHC antigen expression in neurotropic coronavirus infections: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

46 Suzumura, A., Lavi, E., Weiss, S.R. and Silberberg, D.H., Cytokine-induced H-2 antigen expression on oligodendrocytes and astrocytes, *Brain Res.*, 571 (1992) 256–259.

47 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

48 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

49 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

50 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

51 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

52 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

53 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

54 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.

55 Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F., Estrogenic effects on astrocytes: Characterization of the H-2-inducing soluble factor elaborated by infected brain cells, *J. Immunol.*, 140 (1988) 2068–2072.