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Induction of Intracellular Ceramide by Interleukin-1β in Oligodendrocytes

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Abstract The sphingomyelin pathway has been implicated in mediating the effect of several extracellular agents leading to important biochemical and cellular changes. The aim of this investigation is to study interleukin-1β (IL-1β) signaling in oligodendrocytes. For this purpose, the CG4 oligodendrocyte cells were differentiated and incubated with IL-1β. This treatment induced a time- and dose-dependent increase of the endocellular ceramide. To mimic the effect of the elevation of endogenous ceramide, the CG4 cells were treated with the ceramide analogue C2-ceramide. Cell survival, measured with the MTT assay, showed that, by increasing the concentration of ceramide, up to 40% of CG4 cells were dying within 6 h, similar data were obtained with the primary differentiated oligodendrocytes. Condensation of chromatin, nuclear fragmentation, and formation of apoptotic bodies indicated that apoptosis was the cause of death. Surprisingly, long-term exposure (72 h) to increasing concentrations of IL-1β, which increases intracellular ceramide, did not induce oligodendroglial cell death. These results show that an increase of intracellular ceramide is not sufficient to induce apoptosis in oligodendrocytes and that IL-1β signaling through the ceramide pathway in these cells can mediate functions other than programmed cell death. J. Cell Biochem. 66:532–541, 1997.

Key words: oligodendrocytes; II-1β; ceramide; sphingomyelin cycle; apoptosis

INTRODUCTION

The initial observation that sphingosine inhibits protein kinase C (PKC) [Hannun et al., 1986] has induced many investigators to analyze in more details the role that sphingolipids and sphingolipid-derived products play in signal transduction [Hannun and Bell, 1989].

Activation of membrane sphingomyelinase by action of a number of extracellular agents induces hydrolysis of sphingomyelin and intracellular increase of ceramide. The ceramide, in turn, acts as second messanger mediating the effect of the extracellular agents on cell growth, differentiation, and apoptosis. A number of targets for the action of ceramide have been identified and some of these include activated protein kinase (CAPK) [Liu et al., 1994], a ceramide activated protein phosphatase (CAPP) [Dobrowsky and Hannun, 1992], and the PKCζ isoform [Lozano et al., 1994; Muller et al., 1995]. Rapid kinetics of activation, ability of ceramide analogues to mimic ligand action, and cell free reconstitution of the cascade support the concept that the sphingomyelin pathway is a second messanger system.

It has been shown that the sphingomyelin pathway can be activated by several pro-inflammatory cytokines and that it is a primary signaling system for tumor necrosis factor-α (TNFα) in HL60 cells and other cell types [Okazaki et al., 1989; Dressler et al., 1992]. Similarly, the sphingomyelin pathway mediates signaling of the IL-1β in the murine T-helper cell EL-4 [Mathias et al., 1993; Kolesnick and Golde, 1994], in human dermal fibroblasts [Ballou et al., 1992], and in insulin-producing cells [Welsh, 1996]. Recent publications have implicated TNF-α, interleukins-1β and -6 (IL-1β, IL-6), and transforming growth factor-β (TGF-β), as paracrine or autocrine mediators in the regulation of intracerebral immune responses as well as in the proliferation, activation and differentiation of neural cells during brain develop-

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ment [Merrill, 1992; Mizuno et al., 1994; Merrill and Jonakait, 1995].

In a chronic inflammatory environment, the expression of cytokines such as TNF-α, IL-1, and IFNγ has been correlated with the induction of experimental allergic encephalomyelites (EAE) and to the progression of multiple sclerosis (MS), as well as to the destruction of newly formed myelin and remyelinating oligodendrocytes [Merrill, 1992; Merrill and Beneviste, 1996], suggesting a dual role for these cytokines. It has been shown that TNF-α is involved in oligodendrocyte cell death [Merrill, 1992; Merrill et al., 1993; Louis et al., 1993; Merrill and Jonakait, 1995], although several studies failed to demonstrate any effect of soluble recombinant TNF-α on oligodendrocytes [Zajicek et al., 1992; Agresti et al., 1996; E Costantino-Ceccarini, unpublished observations).

In exploring the role played by these two cytokines in the oligodendrocyte lineage, we report the results of experiments that show that IL-1β activates the sphingomyelin pathway, generating an increase of intracellular ceramide in the CG4 cell line. Thus, to mimic the effect of the increase of intracellular ceramide, we have incubated oligodendrocytes with the C2-ceramide analogue. In agreement with the observation of Casaccia-Bonnefill et al. [1996], we have found that ceramide induces apoptosis as shown by the MTT survival assay, by the changes of the nuclear morphology, by the nuclear fragmentation and by the formation of apoptotic bodies in the dying cells. We also show that the increase of intracellular ceramide induced by IL-1β is not related to apoptosis, suggesting that the increase of intracellular ceramide observed in oligodendrocytes, as well as in other cell types, is not necessarily correlated with apoptosis, but is involved in multiple biological activities.

METHODS

Cell Cultures

Culture media. OM-5 and OM-6 consist of the same base medium Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO) containing 6 g/L of glucose and supplemented with 30 nM SeO_2, triiodothyronine 15 nM, biotin 10 ng/ml, and 50 µg/ml gentamycin. In addition OM-6 contains 5 µg/ml insulin 25 µg/ml transferrin and 0.35 µM $FeCl_3$ [Raible and McMorris, 1993]. N1 supplement consists of 2 mM glutamine, 10 ng/ml biotin, 50 µg/ml transferrin, 5 µg/ml insulin, 20 nM progesterone, 100 µM putresceine, and 30 nM SeO_2 [Louis et al., 1992].

Oligodendrocytes primary cultures. Cerebra from 3- to 4-day-old Sprague-Dawley rats, after removal of the meninges, were minced and sequentially passed through nylon meshes of 210-, 130-, and 35-µm pore size [McMorris, 1983]. The dissociated cell suspension was plated on 75-cm^2 flasks in OM-5 containing 10% fetal bovine serum (FBS) (Hyclone, Logan, UT); after 7–8 days, the oligodendrocytes and oligodendrocyte precursors were separated from the astrocyte monolayer by shaking overnight at 200 rpm on a rotary shaker. Oligodendrocytes were purified from contaminating astrocytes by immunopanning on plates coated with Ran2 antibodies. Oligodendrocytes precursors were obtained, after removal of astrocytes, by depleting the culture of the $O4^+$ and $O1^+$ oligodendrocytes (by immunopanning on plates coated with specific antibodies) [Barres et al., 1992]. Oligo-progenitors were maintained in OM-6 medium containing 30% conditioned medium from B104 neuroblastoma cells. Cells were plated on poly-D-lysine-coated 96-well dishes at a density of 10,000 cells/well in OM-5 with 5 µg/ml insulin and next day switched to the differentiation medium OM-6 containing 2% FBS.

CG-4 cells. CG-4 were grown on polyornithine-coated dishes in 70% DMEM-N1 containing 30% of condition medium from B104 neuroblastoma cells. Precursor cells were allowed to differentiate in oligodendrocytes by washing the cells twice in Hanks balanced saline solution (HBSS) and then incubating them for 4 days in DMEM-N1 containing 2% FBS.

Immunocytochemistry. To assess the purity of the panned oligodendrocytes and the differentiation state of the CG-4, cells were plated at 10,000 cells/well in 6mm glass coverslips (Nunc Naperville, IL) precoated with polylysine (primary cells), or polyornithine (CG4 cells). Slides were stained with the monoclonal antibodies A2B5, anti-galactocerebrosides (O1), anti-sulfatides (O4), anti-RAN-2 from hybridomas prepared in our laboratory, and anti-MBP (Biogenesis, UK). Affinity-purified FITC-labeled secondary antibodies (Sigma) were diluted as indicated in the legend to the figures. Under these conditions all CG4 cells were $O1^+$ and approximately 50% were MBP^+. After 4

days in the differentiation medium, the primary olgodendrocytes were 100% were $O1^+$ and more than 90% MBP^+.

Lipid treatment. C2-ceramide and C2-dihydroceramide were generously donated by Dr. Hannun (Duke University, NC) or from Matreya (Pleasent Gap, PA). Lipids were dissolved in 95% ethanol at a stock concentration of 10 mM. The stock solution was diluted in the culture medium and added to the cultures at a concentration within the range of 1–12 μM.

MTT survival assay. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromid) survival assay was performed with the cell viability kit (Boehringer Mannheim, Germany) following the manufacture's instructions. Briefly, 10,000 cells/well were plated in 96-well microtest tissue culture plates coated with either polyornithine or polylysine in 250 μl of the appropriate medium. At the end of the incubation period with the lipids or the cytokine, 150 μl of the medium was removed and 10 μl of MTT solution was added to each well. The plates were incubated for 4 h in the incubator; then, 100 μl of the solubilization solution was added. The plates were incubated overnight at 37°C in humidified atmosphere and then read in an microtiter plate [enzyme-linked immunosorbent assay (ELISA)] reader. The absorbance of the formazan product was determined with a 595-nm filter and the reference wavelength was 655 nm. Viable cells with active mitochondria cleave the tetrazolium ring into a visible dark blue formazan reaction product.

Diacylglycerol kinase assay. Lipids were extracted from controls and cells treated with IL-1β and diacylglycerol kinase assay [Van Veldhoven et al., 1992] was carried out for each point in triplicates. The lipid aliquots were dried under N_2 and resuspended in 20 μl of a solution containing octyl-β-glucoside 7.5%, 5 mM cardiolipin and 1 mM DTPA pH 6.6 and sonicated for 2 min. To the samples were added 60 μl of a buffer solution containing imidazole 100 mM, 100 mM NaCl, 25 mM $MgCl_2$, 2 mM EGTA, 0.4 mM dithiotreitol, and 5 μl sn-1,2-diacylglycerol kinase (Calbiochem, San Diego, CA) diluted in 5 μl of DTPA 1 mM and imidazole 10 mM pH 7.4. Samples were incubated for 10 min at room temperature and then 10 μl of 6 mM [γ-^{32}P]-ATP (spec act ± 50,000 cpm/nmol) was added to the incubation mixture. The enzymatic reaction was carried out at 25°C for 30 min and ceramide-1-phosphate quantitated as previously described [Van Veldhoven et al., 1992]. The amounts of ceramide synthesized was calculated as picomoles/nmol phosphorus [Van Veldhoven and Bell, 1988].

Hoechst. Cells plated at 10,000 cells/wells in coverslips were treated with lipids or cytokines at the indicated concentration. At the end of the incubation the cells were washed 3× in phosphate-buffered saline (PBS), fixed methanol for 5 min at −20°C. The cells were then washed 3× in PBS and incubated in Hoechst 33258 at a concentration of 80 ng/ml in PBS for 10 min at room temperature. The coverslips were then washed in PBS 3×, mounted and observed in a Leitz Aristoplan light microscopy.

Scanning electron microscopy. Cells were plated at 40,000 cells/13-mm glass coverslips coated with polylysine, or polyornothine, and incubated as indicated. The cells were washed 3× in PBS and then fixed for 1 h in Karnowsky buffer (paraformaldehyde 1%, glutaraldehyde 5% in cacodilate buffer 0.2 M pH 7.4), and washed 3× in PBS. The coverslips were dehydrated in increasing concentrations of ethanol, critical point dried and observed in a scanning electron microscope Philips 500.

RESULTS

Cultures of differentiated CG4 cells were treated with recombinant IL-1β, and the concentration of intracellular ceramide was measured in the treated and control cells, by conversion to ceramide-1-phosphate, using the diacylglycerol kinase assay. CG4 cells were incubated with IL-1β at the concentration of 10 ng/ml as a function of time. A low but detectable increase of intracellular ceramide was detected after 10-min incubation, increasing with time and reaching a maximum level within 1 h, when the endogenous ceramide level increased from 19.5 ± 4 pmol/nmol phosphorus of the control cultures to 36 ± 8 pmol/nmolP of the treated cells. After 2-h incubation, the level of ceramide in the treated cells decreased and remained unchanged for the next 2 h (Fig. 1A).

Incubation of CG4 cells with increasing concentrations of IL-1β induced a dose dependent increase of the intracellular ceramide. A small but significant increase ($P < 0.05$) of intracellular ceramide was already detectable at the lowest concentration (0.01 ng/ml) of Il-1β used. Higher concentrations (10–50 ng/ml) induced a further increase in the level of ceramide with maximal effect at 25 ng/ml when the ceramide content increased from a base line of 11.8 ±

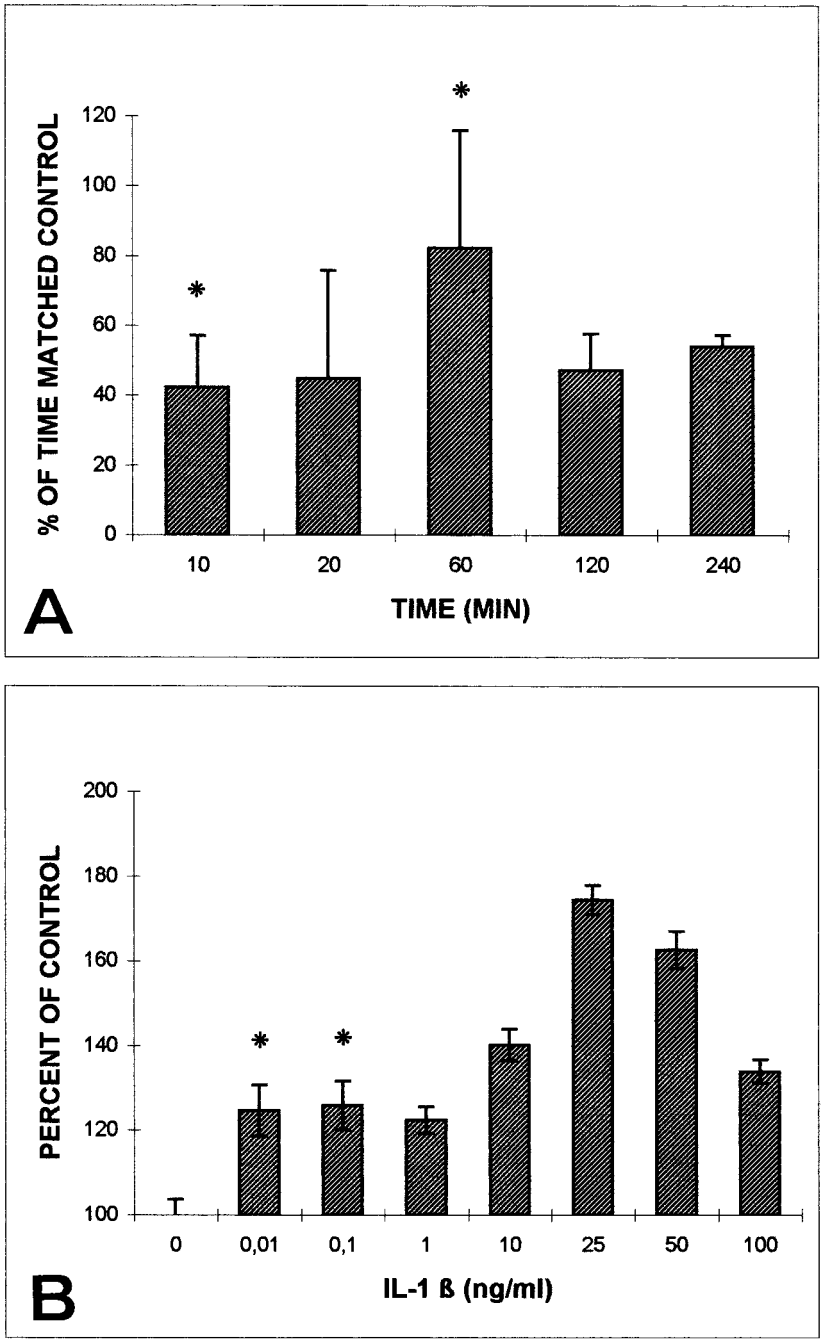

Fig. 1. Increase of intracellular ceramide in differentiated CG4 after treatment with IL-1β. **A:** Ceramide time course. **B:** Dose response. Differentiated cells were incubated with 10 ng/ml of IL-1β and stimulated for the indicated times **(A)** or for 60 min with increasing concentrations of IL-1β **(B).** After lipid extraction, ceramide was quantitated enzymatically with the use of the Escherichia Coli diacyglycerolkinase. Each point represents the mean ±SEM from two experiments run in triplicates. Data represent percentage of time-matched control **(A)** and percentage of the control at 60 min. **(B)**. $*P < 0.05$ with respect to zero time and to its own control (10 min value) **(A),** and to the control at zero time and at 60 min **(B).**

0.49 pmol/nmolP to 25 ± 2 pmol/nmolP in 1 h (Fig. 1B). These data indicate that treatment of CG4 cells with IL-1β induces the activation of the sphingomyelin pathway and increases the intracellular ceramide in a time- and concentration-dependent fashion.

Since the generation of endogenous ceramide activates distinct pathways in different cell types, the cell-permeable C2-ceramide analogue was used to mimic the effect of the raised concentrations of ceramide induced by IL-1β. CG4 precursor cells and primary oligodendro-

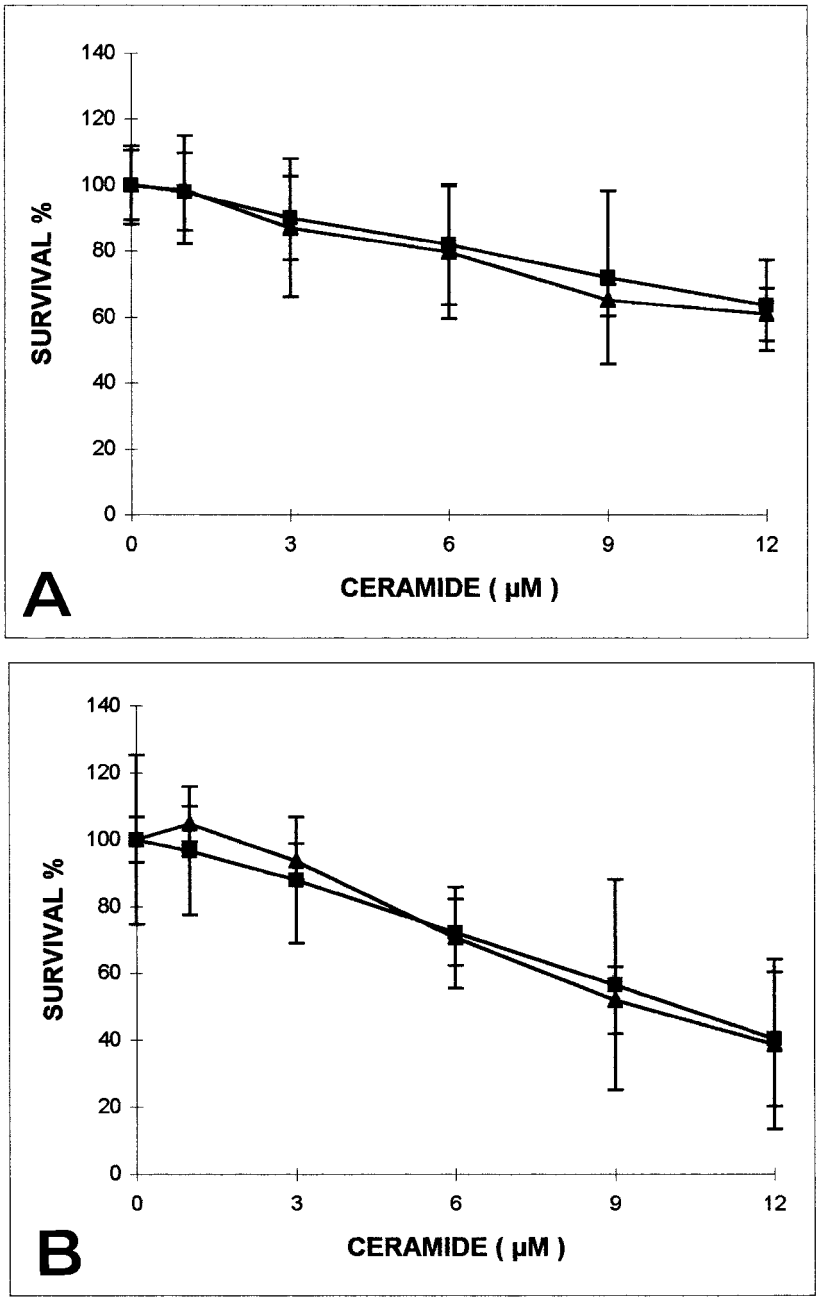

Fig. 2. Survival of oligodendrocytes after 6-h treatment with increasing concentration of C2-ceramide. ■—■, primary oligodendrocytes, ▲—▲, CG4. **A:** Differentiated oligodendrocytes. **B:** O2-A progenitors. 10,000 cells/well were plated in 96-well tissue colture plate and cultured in 2% FBS (differentiated cells) or in N1 medium containing 30% of B104 condition medium (O2-A progenitors). Cells were treated with increasing concentrations of C2-ceramide for 6 h and cell survival assayed as MTT activity. Each point represents the mean of eight determinations of two separate experiments (error bars are ± SEM).

cytes were plated in 96-well microtest culture plates, differentiated in mature oligodendrocytes for 4–5 days and treated for 6 and 24 h with exogenous C2-ceramide at concentrations ranging from 1 to 12 µM. Cell survival was monitored using the MTT assay. Comparison of the kinetics of the C2-ceramide action between the CG4 cells and primary oligodendrocytes (Fig. 2A) indicates that both cell types behave similarly. After 6-h treatment at a concentration of ceramide of 1–3 µM, up to 80–90% of the cells were still viable, while at a concentration of 9–12 µM, a drop in the viability of the cells was observed, although 60–65% of the cells

were still MTT positive (Fig. 2A). The number of viable cells continued to decrease; after 24 h, only 20% of the cells had survived the treatment (data not shown). Oligodendrocyte progenitors were also affected by incubation with C2-ceramide (Fig. 2B). In fact, after 6-h incubation with increasing concentration of C2 ceramide, a slow but consistent loss of cell viability was observed by the MTT assay. As shown, for differentiated oligodendrocytes and CG4 cells, no significant differences were observed in the response to C2-ceramide treatment between immunopanning purified primary and CG4 progenitors. The comparison of the survival pattern of the progenitors and differentiated CG-4 cells, shows that the progenitor are more susceptible than the differentiated cells, to the action of high ceramide concentrations. In fact, after 6-h treatment of 12 μM ceramide, 60% of the differentiated oligodendrocytes survived compared to 40% of the progenitors. Similar doses of C2-dihydroceramide, which differs from C2-ceramide for the presence of a double bond in the 4–5 position of sphingosine had no effect [Hannun, 1994]. The death of oligodendrocytes induced by treatment with exogenous C2-ceramide has been recently reported [Casaccia-Bonnefil et al., 1996]. However, we been unable to repeat the results using the MTT survival assay. Our data do not show the massive cell death reported by these investigators after 6-h incubation with 10 μM C2-ceramide. These differences can be accounted for by the different culture conditions and by the cell death evaluation methods. Our cells were maintained in 2% FCS, and cell survival was determined with the MTT assay, while Casaccia-Bonnefil et al. [1996] were counting the number of $O1^+$ cells.

To determine whether the death induced by exogenously added ceramide is due to apoptosis, primary differentiated oligodendrocytes were incubated for 6 h with 10 μM ceramide and then stained with the antigalactocerebroside antibodies or processed for scanning electron microscopy. Combining these two techniques, we show that oligodendrocytes undergo morphological changes, ranging from the retraction of the cellular processes to the formation of apoptotic bodies. Retraction and fragmentation of the processes were the most frequent changes observed by immunofluorescence (Fig. 3B,C) and by scanning electron microscopy (Fig. 4B,C). The formation of apoptotic bodies and the cell fragmentation indicate that the oligodendro-

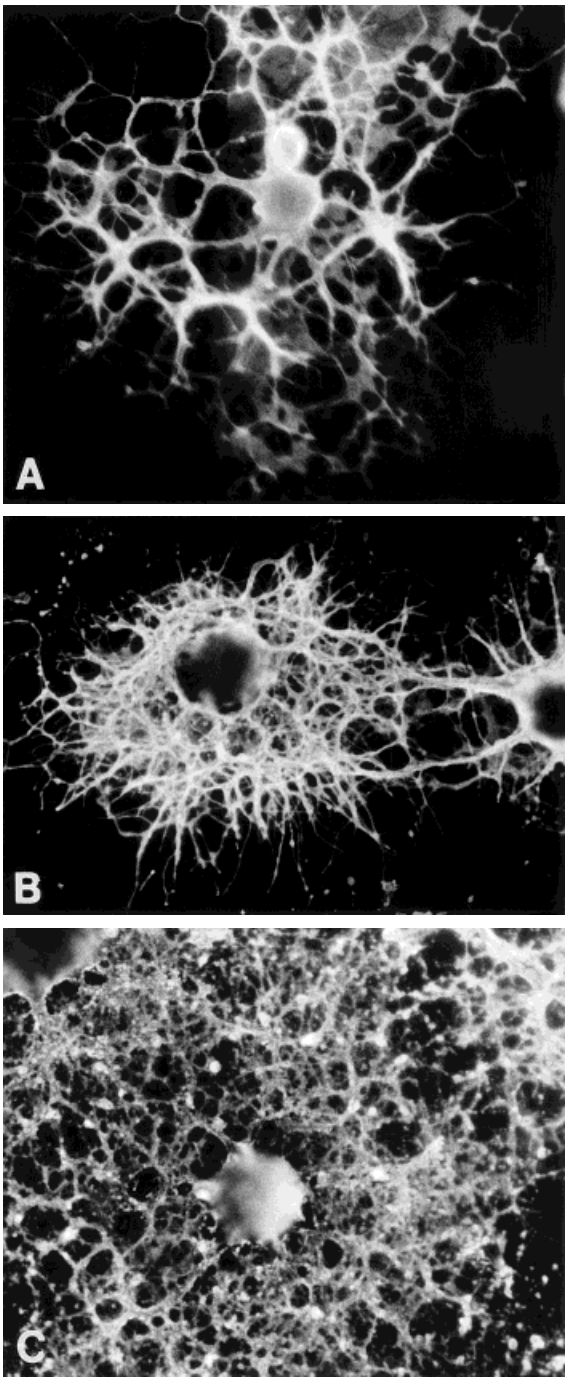

Fig. 3. Primary oligodendrocytes morphology following treatment with 10 μM ceramide for 6 h and stained with O1 monoclonal antibodies diluted 1:2 and the secondary rabbit antimouse IgM diluted 1:140. Control cultures **(A).** In treated cultures cells retraction and fragmentation of the processes are evident (**B** and **C** respectively) ×625.

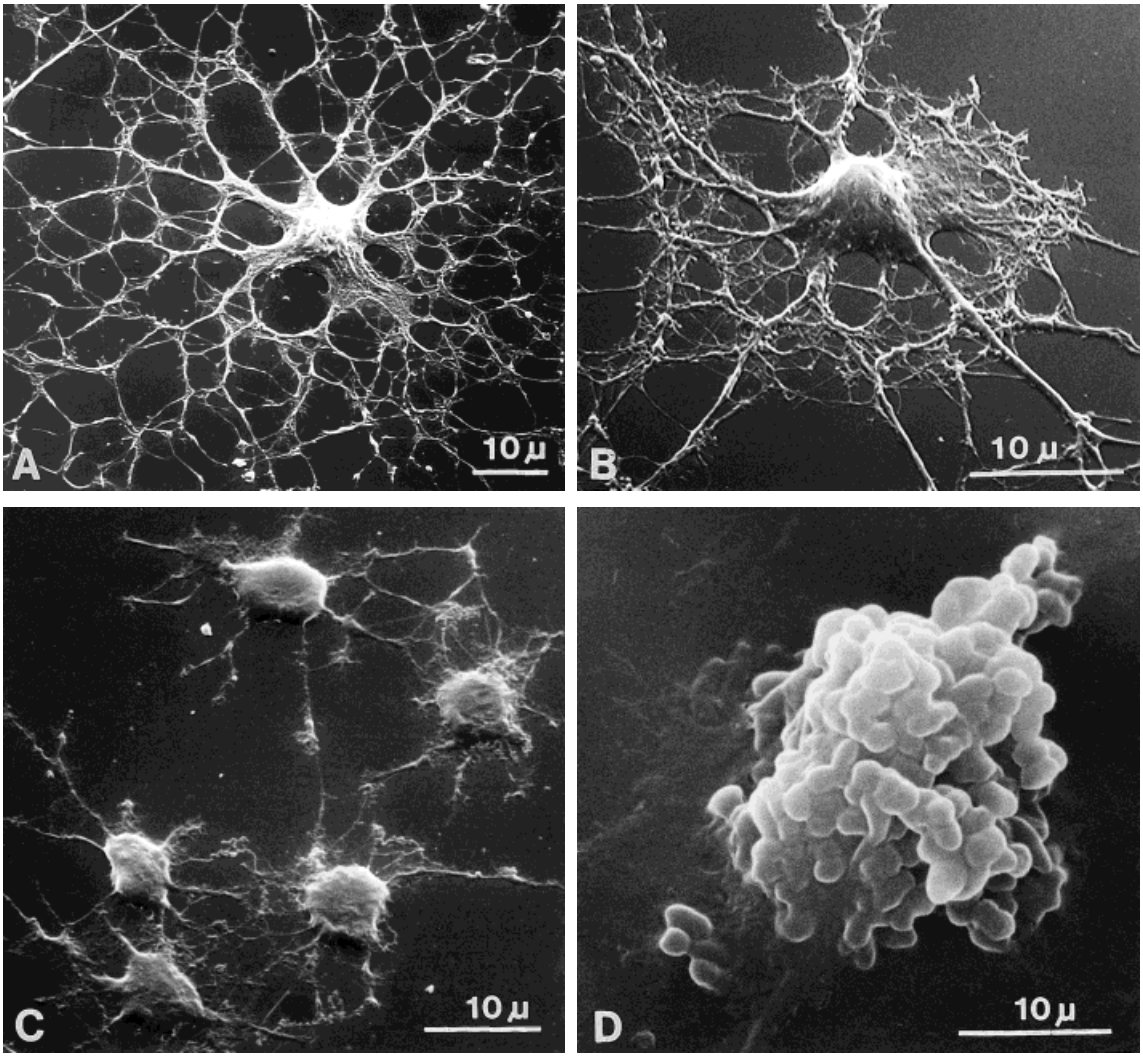

Fig. 4. Scanning electron microphotographs of primary oligodendrocytes after treatment for 6 h with 10 µM ceramide. Control cells **(A)**; treated cells show retraction of the processes **(B,C)** and blebbing of the membrane **(D).**

cytes were in advanced state of apoptosis (Fig. 4A–C).

Staining of the cellular processes with anti-galactocerebroside antibodies was evident even when nuclear changes were already taking place (not shown). It should be noted that incubation of the cells in medium containing 3 µM ceramide also caused loss of the cellular processes, although the mitochondrial function, measured by the MTT survival assay was maintained (data not shown). The Hoechst dye showed uniform staining of the nuclear surface in the control cells (Fig. 5A). In the treated cells, condensed chromatin masses were evident at the periphery of the nucleus (Fig. 5B and upper insert). Nuclear fragmentation and condensed chromatin masses blebbing off from it were also present (Fig. 5B lower insert).

To determine whether the increase of intracellular ceramide induced by IL-1β resulted in cell death, primary oligodendrocytes or CG4 cells were treated with increasing concentrations (0.01–50 ng/ml) of IL-1β for 4–72 h. No significant changes of cell survival or cell morphology of the differentiated primary cultures stained with anti-galalactocerebrosides antibodies were observed.

To explain these results, two possibilities were taken into consideration: (1) prolonged exposure time to ceramide is important in inducing cell death, and (2) activation of NF-κB induced by IL-1β could be protective against apoptotic killing. The activation and the nuclear translocation of NF-κB have been recently shown to have an antiapoptotic effect [Beg and Baltimore, 1996; Van Antwerp et al., 1996]. To test

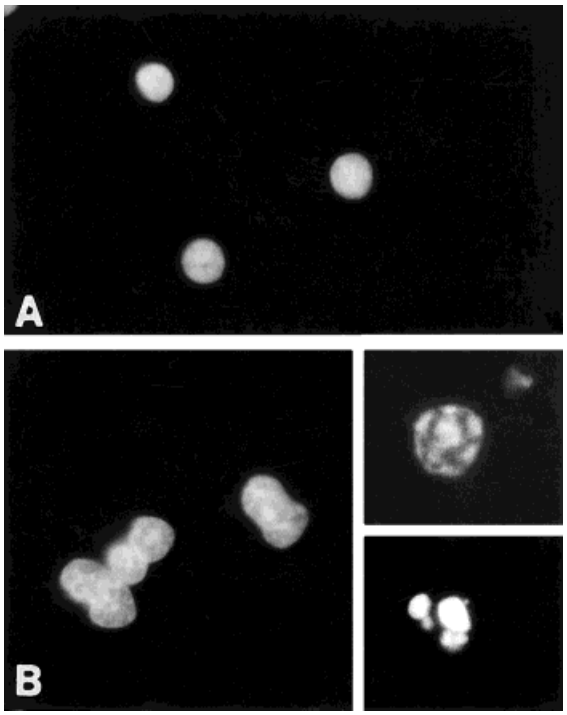

Fig. 5. Nuclear morphology of primary oligodendrocytes following treatment with 10 μM ceramide for 6 h. Cells were grown on coverslips fixed and stained with the Hoechst dye. **A:** Control cells. **B:** Treated cells show chromatin condensation **(B)** ×560. More clearly visible at higher magnification (upper insert, ×1125) and nuclear fragmentation (lower insert × 1125).

the first hypothesis, primary differentiated oligodendrocytes were treated with 12 μM ceramide for 5–60 min. Plates at 5, 10, 15, and every 15 min thereafter were washed once with the OM6 medium containing 5% FCS and once with medium containing 2% FCS and allowed to recover for 24 h. Cell survival was monitored with the MTT assay. At the end of the additional 24-h incubation in fresh medium, it was evident that the effect of ceramide is irreversible; in fact, washing the ceramide was not sufficient to rescue oligodendrocytes from cell death. It was also clear that cell death is dependent on the length of exposure time to ceramide, since the drop in cell survival increased from 5 to 60 min, comparable to that of a parallel set of cultures that had undergone the same treatment with ceramide but were not allowed to recover. Pretreatment of oligodendrocytes with 10 ng/ml of IL-1 for 5 h did not block the subsequent killing induced by ceramide.

DISCUSSION

The contribution of the inflammatory cytokines, IL-1β and TNF-α, to the development and progression of central nervous system (CNS) disease is well enstabilished [reviewed by Merrill and Beneviste, 1996]. These cytokines are known to cause damage to the blood-brain barrier, inflammation, intracerebral immune responses, and demyelination. Several studies have shown that TNF-α, in vitro, induces oligodendrocytes cell death [Merrill, 1992; Merrill et al., 1993; Merrill and Jonakait, 1995; Louis et al., 1993], although other studies [Zajicek et al., 1992; Agresti et al., 1996], including our own unpublished observations, failed to demonstrate any effect of this cytokine on oligodendrocytes. IL-1 has a very wide spectrum of functions involved in cell growth, differentiation, and the process of inflammation. It is active on a variety of target tissues, ranging from vascular endothelium to immunocompetent cells, and to the CNS. It is also synthesized by a variety of different cell types, and in the brain, by neurons and glial cells [Mizuno et al., 1994; Woodroofe, 1995]. IL-1 and TNF have been associated to normal development of the brain, to its pathology, to neural cell migration, proliferation, and death. The effect of IL-1 on oligodendrocytes results in an increased expression of ICAM-1 [Satoh et al., 1991] and heat shock proteins [D'Souza et al., 1994]. IL-1β and TNF-α are known to mediate their effects, in a number of non-neural cell lines, by activating the sphingomyelin cycle and inducing an increase of intracellular ceramide [Ballou et al., 1992; Mathias et al., 1993; Hannun and Bell, 1993; Welsh, 1996].

In this study, we have demonstrated that IL-1β induces an increase of endogenous ceramide, indicating that the sphingomyelin pathway mediates signaling of IL-1β in oligodendrocytes. The shape of the dose-response curve shows a biphasic pattern in which an initial, rather shallow, increase of the endogenous ceramide is followed by a peak. The existence of high- and low-affinity receptors for IL-1β has been established [Lowenthal and Macdonald, 1986], suggesting an explanation for this result. High-affinity receptors may bind at the lower concentration, while lower-affinity receptors bind at the higher concentration of IL-1β. In this context, it is interesting to note that the shape of the time course curve shows an increase ($P < 0.05$) of the endogenous ceramide already after 10 min of treatment, followed by a peak. This pattern may also reflect the presence of two types of IL-1β receptors. Our results

are in agreement with the rapid transient increase of ceramide shown by Welsh et al. [1996] in the insulin producing cell line RINm5F treated with IL-1β.

Oligodendrocytes incubated with exogenous ceramide show chromatin condensation, nuclear fragmentation, and plasma membrane blebbing—the characteristic alterations of apoptotic cells—considered the most rigorous standards for the definition of programmed cell death. These data confirm and extend the results of Casaccia-Bonnefill et al. [1996]; however, we have not observed the extensive cell death reported by these investigators after 6-h incubation with ceramide. This difference can be attributed to the different experimental conditions used. We have, in fact, chosen to incubate differentiated oligodendrocytes and CG4 cells in the presence of 2% serum to prevent the adverse effects that may follow the depletion or limiting concentration of growth factors. Competition for limiting amounts of survival factors is known to induce apoptosis in oligodendrocytes [Barres et al., 1992, 1993; Grispan et al., 1993; Yasuda et al., 1995]. Our experiments have also shown that the cellular effects induced by the endogenous increase of ceramide is dependent on the length of time the cells have been in the presence of ceramide and that these effects are not reversible.

The increase of intracellular ceramide induced by IL-1β in oligodendrocytes does not lead to cell death within 72 h of treatment, the longest time point we have examined. Nuclear translocation of NF-κB induced by IL-1 has been demonstrated to have an anti apoptotic effect [Beg and Baltimore, 1996] in fibrosarcoma apoptotic cell death induced by TNFα. Preincubation with IL-1 does not provide protection against apoptotic cell death induced by ceramide in oligodendrocytes. Whether IL-1 induces activation and traslocation of NF-κB in oligodendrocytes remains unknown and is now under investigation in our laboratory.

Elevation of ceramide has not always been correlated to apoptosis, but it has also been implicated in other biological functions, such as differentiation and cell cycle arrest [Okazaki et al., 1989; Jayadev et al., 1995]. Induction of ceramide by IL-1β in oligodendrocytes could be the trigger of different functions, depending on the stage of differentiation and on the metabolic state of the cells.

In summary, our data show that ceramide alone leads oligodendrocytes to apoptosis, while the increase of intracellular ceramide induced by IL-1 does not, suggesting that different molecular mechanisms are activated by ceramide under the two different experimental conditions through distinct signaling pathways that are largely unknown.

Expression of IL-1 mRNA has been reported in mouse cerebral cortex and other brain regions during development, with neurons and glia being the major sources of IL-1 [Mizuno et al., 1994]. Expression of IL-1 mRNAs under basal conditions is very low; however, following stimulation with lipopolysaccharide (LPS), the mRNAs are strongly increased in all the brain regions tested [Gabellec et al., 1995]. It is well documented that IL-1 and TNF-α are involved in inflammation of the CNS and are elevated in multiple sclerosis. In conclusion, it appears that at lower concentrations IL-1 may in fact be involved in normal development of the nervous system, while following brain trauma or an inflammatory response, the overproduction of this and other cytokines may result in a homeostatic imbalance and contribute to the outcome of the pathologic event.

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