Expression of a complete and functional complement system by human neuronal cells \textit{in vitro}

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Abstract

We demonstrate \textit{in vitro} expression of complement components, i.e. C3, factor H (FH), factor B (FB), C4, C1-inhibitor (C1-inh), C1q, C5, C6, C7 and C9, by four human neuroblastoma cell lines IMR32, SKNSH, SH-SY5Y and KELLY. Activating proteins C4, C9 and C1q, and regulatory proteins FH and C1-inh were produced constitutively by the four cell lines. C3, C6 and FB were mainly produced by SKNSH and SH-SY5Y. Western blot experiments showed that secreted proteins were structurally similar to their serum counterparts. An additional polypeptide of 43 kDa with FH immunoreactivity was detected, which could correspond to the N-terminal truncated form found in plasma. Regulation of complement expression by inflammatory cytokines, lipopolysaccharide and dexamethasone was tested \textit{in vitro}. These factors had no significant effects on activating synthesis of components C3, FB and C4, but expression of regulating components C1-inh and FH was strongly increased particularly by IFN-\(\gamma\) and tumor necrosis factor-\(\alpha\). The rate of synthesis of complement components was dependent on the differentiation of neuroblastoma cells. This effect of differentiation was also observed on normal rat neurons. Rat cerebellar granule cells constitutively expressed mRNA for C4 and C1q, but expression of C3 mRNA was induced by differentiation. This study shows that neurons could be another local source of complement in the brain, besides astrocytes and microglia. Human neuroblastoma cell lines can constitute an interesting model to analyze complement biosynthesis by human neurons. Local complement expression by neurons \textit{in vivo} may be implicated in some physio-pathological processes.

Introduction

The complement system is composed of >20 plasma and associated cell membrane proteins, and is an important pro-inflammatory element of the innate immune system. Complement may be activated by one of the two activation cascades (alternative or classical pathway) that leads to the formation of the C5b–9 cytoplytic membrane attack complex (for review, see 1). The complement system plays a major role in host defense against microorganisms, and in the processing and elimination of immune complexes. Fragments produced during complement activation mobilize and activate phagocytic cells by anaphylactic and chemotactic activities, and opsonize foreign particles and damaged cells leading to destruction by phagocytosis (for review, see 1,2).

Although the liver is the primary site of synthesis of complement components, numerous extra-hepatic sources of complement have been shown. These includes monocytes/macrophages (3,4), fibroblasts (5,6), epithelial cells (7–9), keratinocytes (10,11), endothelial cells (12–15), adipocytes (16,17), osteoblastic cells (18,19) and skeletal myoblasts (20–22). This extra-hepatic synthesis of complement plays an
important role in local inflammatory processes but complement presents new functions which are not connected with immunity and which are specific of the tissue where complement is expressed (for review, see 23).

Evidence implicating complement in demyelination, neurodegeneration and other central nervous system (CNS) pathologies has accumulated during the last few years (for review, see 24). Cells in the brain are normally protected from serum complement by the blood-brain barrier, but complement may be synthesized intrathecally, raising the possibility that endogenous complement might damage surrounding neurons and oligodendrocytes, and contribute to pathology in inflammatory CNS diseases. Complement biosynthesis in the CNS is now well established. Microglia (25–27), astrocytes (28–33) and oligodendrocytes (34) are all sources of complement in the brain. Moreover, neurons have been suggested to be a potential source of complement. Complement C4 mRNA was detected by in situ hybridization in neurons in a model of experimental rat brain lesioning (25,26). Walker and McGeer detected C4 and C3 mRNA in neuroblastoma cell lines by RT-PCR (35), and expression of the classical complement pathway was recently observed by in situ hybridization and immunohistochemistry in post-mortem Alzheimer’s disease brain tissue (36,37).

It is quite difficult to obtain and maintain pure cultures of neurons from human brains. To get round this problem, well-characterized human neuroblastoma cell lines can be used as a model for human neurons. Using this model, we have previously shown that human neuroblastoma cells have the capacity to spontaneously activate the classical pathway of complement in serum, but with moderate cell killing, despite the deposition of activation complement fragments on these cells (38). Neuroblastoma abundantly expressed complement inhibitors membrane cofactor protein (MCP) and CD59, but not decay accelerating factor (DAF) and complement receptor 1 (CR1) (38).

To enlarge the relationship between neurons and the complement system, we wanted to observe if neurons had the capacity to express a complete complement system. In this paper, we demonstrate the expression of components of the alternative, classical and terminal pathways of the complement system, i.e. C3, factor H (FH), factor B (FB), C4, C1-inhibitor (C1-inh), C1q, C5, C6, C7 and C9, by neuronal cells and their regulation by different factors.

Methods

Chemicals, cytokines and antibodies

Lipopolysaccharide (LPS) purified from *Escherichia coli*, biotinamidocaproate *N*-hydroxysuccinimide, and proteinase inhibitors benzamidine, pepstatin A, leupeptin, EDTA and EGTA were from Sigma-Aldrich Chimie (St Quentin Fallavier, France). Recombinant IFN-γ (RU42369, sp. act. 2×10^7 U/mg) was a gift from Roussel Uclaf (Paris, France). Recombinant IL-1β (10^8 U/mg) and recombinant tumor necrosis factor (TNF)-α (10^2 U/ml) were obtained from the Institute of Highly Pure Biopreparations (St Petersburg, Russia). Recombinant IL-6 (2×10^7 U/ml) was from Boehringer Mannheim (Meylan, France). Dexamethasone (DXM), sodium phosphate salt (sterile, apyrogenic solution for human therapeutic use) was from Merck, Sharp, Dohme-Chibret (Paris, France). The anti-FH mAb OX24 were a gift of Dr R. B. Sim (Medical Research Council Unit, Oxford, UK). Monospecific polyclonal anti-human FB, C4 and C1-inh antibodies were from Atlantic Antibodies (Stillwater, MN). Monospecific polyclonal anti-FH and C3 antibodies were obtained by immunization of rabbits with the respective purified human proteins, and were purified by affinity chromatography. Polyclonal anti-C3, FH, FB, C4 and C1-inh antibodies were biotinylated and used as second antibodies in ELISA. Peroxidase-conjugated avidin and alkaline phosphatase-conjugated avidin were obtained from Sigma.

Cell cultures

Three human neuroblastoma cell lines (IMR32, SKNSH and KELLY) were obtained from ATCC (Rockville, MD). Human neuroblastoma cell line SH-SY5Y was generously provided by Dr A. Delacourte (INSERM U422, Lille, France). Cells were cultivated in DMEM medium (Sigma) containing 10% heat-inactivated FCS (Sigma), 2 mM L-glutamine (Biowhittaker, Fontenay-sous-Bois, France), 100 IU/ml penicillin (Biowhittaker) and 100 µg/ml streptomycin (Biowhittaker) in 75 cm² flasks (Falcon, Poly-Labo, Strasbourg, France), and grown at 37°C in a humidified incubator containing 5% CO₂/95% air. The culture medium was changed every 48 h. Cells were dispensed for culture passages with 0.05% trypsin/0.02% EDTA in PBS without Ca²⁺/Mg²⁺ (Sigma). Cells were then centrifuged at 200 g and the pellets were dispersed in the culture medium. The cell lines were routinely screened by the Hoechst 33258 DNA staining method to ensure that they were mycoplasma-free.

Cells were stimulated with different concentrations of cytokines (20–1000 IU/ml), DXM (10⁻⁷–10⁻⁵ M) and LPS (0.5–6.0 µg/ml) for various periods (6–72 h). For quantification in all experiments, cells were removed by gentle scraping or by brief trypsinization and counted.

Immature granule cells were obtained from cerebelli of 8-day-old Wistar rats and cultured in a chemically defined medium as previously described (39). Differentiated granule cells were cultured in a DMEM medium supplemented with 25% Ham’s F12 medium, 10% FCS, 25 mM KCl and 1% of an antibiotic–antimycotic solution (10000 U/ml penicillin, 10 mg/ml streptomycin and 25 µg/ml fungizone). Then 10 µM cytosine-arabinofuranside (Sigma) was added in the culture medium 24 h after plating, in order to eliminate non-neuronal cells. Differentiated granule cells were used after 10 days in vitro.

Western blotting experiments

Confluent cells were intensively washed with apyrogen 0.9% NaCl and cultivated in DMEM medium without FCS for 48 h. Supernatants were collected, centrifuged at 400 g for 10 min to remove cellular debris, mixed with a cocktail of proteinase inhibitors (1 mM EDTA, 1 mM EGTA, 5 mM benzamidine, 10 µg/ml leupeptin and 5 µg/ml pepstatin A), dialysed overnight against buffer (50 mM Tris/10 mM EDTA) and concentrated 20 times by lyophilization. The powder was dissolved with 1 volume of distilled water and 1 volume of Laemmli buffer (2% SDS, 10% glycerol and 250 mM Tris-HCl, pH 6.8). This concentrate was analyzed by SDS-PAGE.
according to the method of Laemmli (40) in non-reducing conditions. Then, the proteins were electrotransferred onto a nitrocellulose sheet (Gelman, Ann Arbor, MI) at 6 V/cm for 2h, as described by Towbin et al. (41). Prestained protein markers from Sigma were used as mol. wt standards.

Blots were saturated in 0.2% BSA/0.05% gelatin in PBS and then incubated with a specific polyclonal biotinylated antibody diluted in saturation buffer. After extensive washings, the peroxidase-conjugated avidin diluted in saturated buffer was added to the nitrocellulose sheet. Finally, the blots were developed with the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham, Les Ulis, France).

ELISA
Cells were cultivated on 24-well plates (Falcon), and, after reaching confluence, washed with 0.9% NaCl and 500 µl of DMEM medium with 10% FCS, with or without stimulating factors, added per well. The whole supernatant from unstimulated or stimulated-cells were collected at 6, 12, 24, 30, 48 and 72 h, treated with a mixture of proteinase inhibitors, and frozen at ~70°C until used.

Complement proteins were quantified in cell culture supernatants by ELISA in 96-well microtiter plates (Viron, Roche Products, Rosny-sous-Bois, France), as previously described (20). For each assay, a standard curve was established with known amounts of a calibrated pool of normal human sera.

Statistical analysis
The significance of difference between values was estimated by Student's t-test. P < 0.05 was considered to show statistically significant differences.

RNA preparation and RT-PCR
Total RNA from culture of neuroblastoma cell lines was obtained by the guanidium isothiocyanate procedure (42) and ultracentrifugation on cesium chloride gradients. Total RNA from rat cerebellum granular primary cell culture was prepared using the RNeasy extraction kit according to the manufacturer's instructions (Qiagen, Courtaboeuf, France). The purity and yield of total RNA were determined spectrophotometrically by measuring the absorbance of an aliquot at 260 and 280 nm. RNA preparation was checked by agarose gel electrophoresis.

RT-PCR was performed according to the protocol of Kawasaki (43), using 2 µg of total RNA, primer pd(N)6 (Pharmacia, St Quentin-en-Yvelines, France), and MMLV reverse transcriptase (Gibco/BRL, Life Technologies, Cergy-Pontoise, France) for the reverse transcription reaction and specific 20mer oligonucleotide primers (Genosys, Cambridge, UK) for PCR in a Hybaid Omnigene (Céra-Labo, Aubervilliers, France) DNA thermal cycler (30 cycles) (detailed protocol on request). Amplification of specific cDNA was confirmed on agarose gels staining with ethidium bromide for UV detection.

Different controls were performed (no RNA, no MMLV reverse transcriptase or no reverse transcriptase product) to ensure the validity of the reaction product. Primer positions (upstream and downstream) are given according to their migrated location on one of the truncated forms of human FH found in plasma (position (nucleotide number in the cDNA sequence from 5' to 3'). The EMBL accession numbers, amplified sequences and expected product sizes were as follows: C3: K02765, 2456–2690, 234 bp; FH: K07523, 334–826, 492 bp; FB: K01566, 1032–1590, 558 bp; C4: K02403, 515–772, 257 bp; C1-inh: M13203, 51–401, 350 bp; C1q: X03084, 94–531, 437 bp; C5: M57729, 3901–4300, 399 bp; C6: J05024, 2041–2437, 396 bp; C7: J03507, 2401–2790, 389 bp; C9: X02176, 1361–1771, 410 bp; rat C3: M29866, 172–609, 437 bp; rat C1q: X71127, 333–792, 459 bp.

Functional tests
Functional activities of C3 and FH were determined as described (31). Briefly, FH activity was estimated by hemolytic C3 assay. Activities of FH were investigated by their capacity to cleave the α chain of C3b (cofactor assay). The cleavage of 125I-labeled C3b by FH was analyzed by electrophoresis and autoradiography. The percent of residual C3b α chain was estimated by scanning autoradiograms using a Lecphor camera densitometer coupled to a computer (Biocom, Les Ulis, France).

Results
Kinetics of C3, FH, FB, C4 and C1-inh secretion by SKNSH cells
SKNSH cells at confluence were cultivated in 24-well plates in 0.5 ml medium with 10% FCS. At the indicated time intervals, supernatants were collected and components C3, FH, FB, C4 and C1-inh were quantified by ELISA (Fig. 1). These components were constitutively produced at different rates. C3 and FH were expressed at a rate of 48.1 ± 6.1 and 61.4 ± 8.6 ng/10^6 cells/ml in 48 h respectively, whereas C4, FB and C1-inh secretion was <25 ng/10^6 cells/ml in 48 h (22.0 ± 4.2, 12.9 ± 3.0 and 12.0 ± 1.7 respectively). For all components, the secretion was maximum at 24 or 48 h and then reached a plateau.

Characterization of C3, FH, FB, C4 and C1-inh secreted by SKNSH cells
We investigated the presence of complement proteins C3, FH, FB, C4 and C1-inh in human neuroblastoma cell line SKNSH culture supernatants. Supernatants were collected from 48 h cultures without FCS, and analyzed by SDS–PAGE under non-reducing conditions and Western blotting (Fig. 2). These experiments were performed on supernatants from unstimulated or stimulated cell cultures, since it is known that complement secretion is often modified by stimulation with inflammatory cytokines. Supernatants were obtained from unstimulated cells at 48 h (lane 1), cells stimulated with 1000 IU/ml TNF-α (lane 2), 200 IU/ml IFN-γ (lane 3), 100 IU/ml IL-6 (lane 4) or 50 IU/ml IL-1β (lane 5). Diluted human serum was analyzed in parallel as a positive control (lane T).

Polyclonal anti-C3 and anti-C4 antibodies revealed only one band that migrated like serum C3 (180 kDa) and C4 (206 kDa) (not shown) respectively. Polyclonal anti-FH antibody detected two bands of 155 and 43 kDa under basal conditions and stimulation with cytokines. The upper band migrated like serum FH and the lower band could correspond to one of the truncated forms of human FH found in plasma (44,45). Polyclonal anti-FB and anti-C1-inh antibodies
Fig. 1. Kinetics of C3, FH, FB, C4 and C1-inh secretion by SKNSH cells. Cells at confluence (10^5 cells/well) were cultivated in medium with 10% FCS, and after indicated times supernatants were collected and analyzed by ELISA for C3, FH, FB, C4 and C1-inh. Results are expressed as the mean ± SD of duplicate measurements from triplicate cultures.

Fig. 2. Western blot analyses of complement proteins secreted by SKNSH cells. Confluent SKNSH cells (4×10^6 cells/75 cm² culture flasks) were cultivated 48 h without FCS. Supernatants were concentrated 20-fold and analyzed by SDS-PAGE (non-reduced conditions). Proteins were transferred onto nitrocellulose sheets for immuno-detection with biotinylated polyclonal antibodies as described in Methods. Negative controls were assessed for each protein with culture medium alone. Lane 1, unstimulated cells; lane 2, cells stimulated for 48 h with TNF-α (1000 IU/ml); lane 3, cells stimulated for 48 h with IFN-γ (200 IU/ml); lane 4, cells stimulated for 48 h with IL-6 (1000 IU/ml); lane 5, cells stimulated for 48 h with IL-1β (50 IU/ml); lane T, positive control which consisted of a diluted human serum.
Complement biosynthesis by neurons revealed one band of 90 and one of 110 kDa respectively, in serum and neuroblastoma culture supernatants from unstimulated or stimulated cells.

The protocol used for concentration of supernatants precluded an accurate quantitative analysis of these data. Quantitative effects of cytokines on secretion were studied in detail by ELISA (see below).

Functional activity of C3 and FH produced by SKNSH cells

The functional activity of the newly secreted C3 was investigated by hemolytic assay. The sp. act. of C3 produced by SKNSH cells was estimated to be $1.3 \pm 0.5 \times 10^9$ effective molecules/ng of C3. The specific activity of fresh normal serum was determined in parallel and is similar to that of C3 from SKNSH cells (not shown).

FH purified from supernatants of SKNSH cells was functionally active as demonstrated by its capacity to cleave C3b. Anti-FH antibody completely blocked this cleavage (not shown).

Effects of cytokines on regulation of C3, FH, FB, C4 and C1-inh secretion by SKNSH cells

SKNSH cells were cultivated in medium containing different amounts of stimulating factors: IFN-γ, IL-1β, TNF-α, IL-6, LPS and DXM. We determined the kinetics of C3, FH, FB, C4 and C1-inh secretion by ELISA.

Two cytokines appeared to regulate significantly the secretion of FH and C1-inh by SKNSH cells: (i) IFN-γ which increased FH (8-fold; $P < 0.0001$) and C1-inh (4-fold; $P < 0.0001$) secretion, and (ii) TNF-α which increased FH (2.5-fold; $P < 0.0001$) and C1-inh (4-fold; $P < 0.0001$) secretion (Fig. 3). These effects were time dependent with a maximum effect at 48 h and dose dependent (not shown).

The other stimulating factors tested, i.e. IL-1β, IL-6, LPS and DXM, had no effect on the secretion of the five components studied (not shown).

To observe the conjugated effects of cytokines on FH and C1-inh secretion by SKNSH cells, different combinations of cytokines were made (Fig. 3).

Combinations of these different stimulating factors had no significant effects on complement proteins secretion compared to the effects of the cytokine alone. In a general manner, IFN-γ and TNF-α were very slightly enhanced by IL-1β. The combination of TNF-α and IFN-γ gave the higher rate of secretion.

Qualitative analysis of mRNA expression of complement components by SKNSH cells and other neuroblastoma cell lines by RT-PCR

Total RNA were purified from SKNSH cell culture. After reverse transcription, PCR was performed with specific primers to analyze the expression of C3, FH, FB, C4 and C1-inh mRNA. PCR products were electrophoresed on 1% agarose gels in parallel with positive and negative controls of the RT-PCR reactions.

SKNSH cell line expressed mRNA for C3, FH, FB, C4 and C1-inh since amplification products were identified at the correct size by comparison with size markers. Expression of C3, FH, FB, C4 and C1-inh mRNA was detected under basal conditions and after stimulation with 200 IU/ml IFN-γ (Table 1).

In addition, this method showed C1q, C5, C6, C7 and C9 mRNA expression by these cells (Table 1). Specific C1q, C6 and C9 cDNA products were amplified and identified from SKNSH total RNA from culture under basal conditions and after stimulation. Expression of C5 and C7 mRNA was not constitutive but was induced after stimulation with IFN-γ.

Expression of these complement components mRNA was studied from the others neuroblastoma cell lines IMR32, KELLY and SH-SY5Y under the same conditions (Table 1). SH-SY5Y cells expressed constitutively and under stimulation C3, FH, FB, C4, C1-inh, C1q, C5, C6, C7 and C9 mRNA. In IMR32 cell culture, the expression of FH, C4, C1-inh, C1q, C6 and C9 mRNA was constitutive and easily detectable, whereas that of C3 mRNA was very low, C5 mRNA was induced by IFN-γ, and FB and C7 mRNA were never detected.
The complement system is an important element of the innate immune system. It has the capacity to mediate directly cell killing or indirectly through the generation of opsonins including C1q and fragments of C3 and C4. Complement is not only implicated in defense against microorganisms but plays a major role in the elimination of immune complexes and is highly implicated in the inflammation caused by the generation of pro-inflammatory substances. The liver is the major site of complement biosynthesis. Several sites of extra-hepatic synthesis have been reported for complement components. Complement biosynthesis in the CNS by microglia and astrocytes is now well established.

Previous observations (25,26,35–37) mainly related to the expression of mRNA of C4 and C3 or of the classical pathway during Alzheimer’s disease have suggested that cells of neuronal origin have the capacity to express complement components. Our previous works have shown that human neuroblastoma cells have the capacity to spontaneously activate the classical pathway of complement in serum and abundantly expressed complement inhibitors MCP and CD59, but not DAF and CR1 (38). This study described for the first time the expression and regulation of complement proteins by neuronal cells. Using cultured human neuroblastoma cell lines, we clearly demonstrate the expression of three components of the alternative pathway and three of the classical pathway of the complement system: C3, FH, FB, C4, C1q and C1-inh. These syntheses were detected by Western blotting and quantified by ELISA, performed on cell supernatants. Specific mRNA for C3, C4, C1q, FB, FH and C1-inh were demonstrated by RT-PCR, and proved the neuronal origin of these syntheses in vitro. Moreover, specific mRNA for C5, C6, C7 and C9 was also demonstrated by RT-PCR. These results showed that neuronal cells can produce a complete complement system.

The newly synthesized proteins were similar in molecular size and activity to their serum counterparts. For FH, an additional band of 43 kDa was observed in addition to the well-characterized 155 kDa species when analyzed by SDS-PAGE under non-reduced conditions. This 43 kDa species most probably corresponds to the N-terminal truncated form found in plasma (44,45).

Thus, these cells represent interesting models easily available to analyze complement expression by neurons, because they produce two functional activation pathways and, with the synthesis of terminal components, probably lead to the formation of the cytolytic membrane attack complex.

To investigate complement expression by normal neurons in vitro, we analyzed the expression of C3, C4 and C1q mRNA. Undifferentiated rat cerebellum granular primary cells expressed constitutively mRNA for C4 and C1q but no C3 mRNA.

Synthesis of complement components by neuronal cells had a specific feature compared to other cell types producing complement (i.e. macrophages, endothelial cells or glial cells). The most prominent characteristic was the inefficacy of IFN-γ or other cytokines to stimulate the synthesis of C3, C4 and FB. Only synthesis of regulatory proteins FH and C1-inh was highly up-regulated by IFN-γ and TNF-α. This would indicate that neurons respond to inflammation by increasing the secretion of complement regulators to inhibit complement activation.

Another feature but shared by neurons and by monocytic cells was a correlation between the basal rate of complement biosynthesis by neurons, because they produce two functional activation pathways and, with the synthesis of terminal components, probably lead to the formation of the cytolytic membrane attack complex.

Table 1. Expression of complement components mRNA by four neuroblastoma cell lines

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RT-PCR was performed according to the protocol described in Methods, using total RNA from unstimulated cells (NS) or stimulated with 250 IU/ml IFN-γ (IFN). The results are given according to the quantity of each RT-PCR CDNA product detected after ethidium bromide staining (–, not detectable; +, faint and reproducible signal; ++, strong cDNA product; ++++, signal stronger than under NS conditions).

In KELLY cell line culture, the expression of FH, C4, C1-inh, C1q and C9 mRNA was detected constitutively and under stimulations, C6 mRNA was induced by IFN-γ stimulation, and C3, FB, C5 and C7 mRNA were not detected.

These results of mRNA expression observed by RT-PCR have been confirmed by studies on effects of cytokines on complement components secretion by the four cell lines, measured by ELISA (not shown). Moreover, it appears from this last study that each cell line synthesized each protein at its own rate, IMR32 cells being the lowest potent complement producer, and that the four cell lines exhibited the same response to cytokines.

Analysis of complement components mRNA expression by rat cerebellum granular primary cell culture by RT-PCR

To investigate complement expression by normal neurons in vitro, total RNA was purified from undifferentiated or differentiated rat cerebellum granular primary cell culture. After reverse transcription, PCR was performed with specific primers to analyze the expression of C3, C4 and C1q mRNA. PCR products were electrophoresed on 1% agarose gels in parallel with positive and negative controls of the RT-PCR reactions (Fig. 4).

Undifferentiated rat cerebellum granular primary cells expressed mRNA for C4 and C1q since their amplification products were identified at the correct size of 258 and 454 bp respectively, but not mRNA for C3, whereas differentiated rat cerebellum granular primary cells expressed mRNA for C4, C1q and C3. Moreover, these expressions were enhanced when rat cerebellum granular primary cells were differentiated.

Discussion

The complement system is an important element of the innate immune system. It has the capacity to mediate directly cell killing or indirectly through the generation of opsonins but shared by neurons and by monocytic cells was a correlation between the basal rate of complement biosynthesis by neurons, because they produce two functional activation pathways and, with the synthesis of terminal components, probably lead to the formation of the cytolytic membrane attack complex.

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Analysis of complement components mRNA expression by rat cerebellum granular primary cell culture by RT-PCR

To investigate complement expression by normal neurons in vitro, total RNA was purified from undifferentiated or differentiated rat cerebellum granular primary cell culture. After reverse transcription, PCR was performed with specific primers to analyze the expression of C3, C4 and C1q mRNA. PCR products were electrophoresed on 1% agarose gels in parallel with positive and negative controls of the RT-PCR reactions (Fig. 4).

Undifferentiated rat cerebellum granular primary cells expressed mRNA for C4 and C1q since their amplification products were identified at the correct size of 258 and 454 bp respectively, but not mRNA for C3, whereas differentiated rat cerebellum granular primary cells expressed mRNA for C4, C1q and C3. Moreover, these expressions were enhanced when rat cerebellum granular primary cells were differentiated.
Complement biosynthesis by neurons

Fig. 4. Expression of C3, C4 and C1q mRNA by RT-PCR by rat cerebellum granular primary cells. Total RNA were isolated from non-differentiated or differentiated by cytosine-arabinoside treatment rat cerebellum granular primary cell culture. After reverse transcription, PCR was performed with specific primers to analyze expression of C3, C4 and C1q. PCR products were electrophoresed on 1% agarose gels. The gels were stained with ethidium bromide and photographed under UV light. Lane 1, positive control (rat liver RNA); lane 2, as lane 1 without MMLV reverse transcriptase; lane 3, negative control (without RNA); lane 4, non-differentiated cells; lane 5, as lane 4 without MMLV reverse transcriptase; lane 6, differentiated cells; lane 7, as lane 6 without MMLV reverse transcriptase; lane 8, negative control (without reverse transcriptase products).

biosynthesis and the degree of differentiation of neurons. There were significant variations between the basal rate of complement synthesis by the four cell lines studied. These differences may be attributed to the degree of differentiation of the cells because the IMR32 cell line, which was the less differentiated cell line, had the lowest rate of complement components synthesis. However, the four cell lines responded identically to cytokine stimuli. This effect of differentiation was also observed on normal rat neurons. Rat cerebellum granular cells expressed constitutively mRNA for C4 and C1q, but not C3 mRNA. Differentiation of these cells in mature neurons, not only enhanced C4 and C1q mRNA expression, but induced mRNA expression of C3.

The role of complement expression by neurons remains unclear. Complement has also been implicated in several brain disorders, notably demyelination and neurodegeneration. During Alzheimer’s disease, an increase of complement components mRNA was observed and recently was attributed to neurons by in situ hybridization and immunohistochemistry (36,37). Our in vitro data showed that cytokines were not involved in this phenomenon since C3 and C4 synthesis were not up-regulated by inflammatory cytokines (TNF-α, IL-1β, IL-6 and IFN-γ), suggesting that other factors were responsible for complement overexpression during the disease, possibly β-amyloid peptide that was previously shown to enhance C3 expression by microglia (27). During Alzheimer’s disease, complement could participate to neuron loss by different pathways. We previously showed that complement could kill neurons by direct lysis (38). Recently, Farkas et al. (46) induced a cell death program in neurons by a C5a-derived peptide, suggesting that anaphylatoxins may trigger neuron death. The recent observations of anaphylatoxin receptor expression by neurons (47) highlight the biological significance of complement in the brain.

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TNF tumor necrosis factor
myoblasts
FB factor B
component of complement by osteoblastic cells treated with
DAF decay accelerating factor
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Abbreviations

C1-inh C1-inhibitor
CNS central nervous system
CR1 complement receptor 1
DAF decay accelerating factor
DMX dexamethasone
FB factor B
FH factor H
LPS lipopolysaccharide
MCP membrane cofactor protein
TNF tumor necrosis factor

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