Decay-Accelerating Factor (CD55) Is Expressed by Neurons in Response to Chronic but Not Acute Autoimmune Central Nervous System Inflammation Associated with Complement Activation

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Decay-Accelerating Factor (CD55) Is Expressed by Neurons in Response to Chronic but Not Acute Autoimmune Central Nervous System Inflammation Associated with Complement Activation

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There is compelling evidence that a unique innate immune response in the CNS plays a critical role in host defense and clearance of toxic cell debris. Although complement has been implicated in neuronal impairment, axonal loss, and demyelination, some preliminary evidence suggests that the initial insult consequently activates surrounding cells to signal neuroprotective activities. Using two different models of experimental autoimmune encephalomyelitis, we herein demonstrate selective C1q complement activation on neuron cell bodies and axons. Interestingly, in brains with chronic but not acute experimental autoimmune encephalomyelitis, C3b opsonization of neuronal cell bodies and axons was consistently associated with robust neuronal expression of one of the most effective complement regulators, decay-accelerating factor (CD55). In contrast, levels of other complement inhibitors, complement receptor 1 (CD35), membrane cofactor protein (CD46), and CD59 were largely unaffected on neurons and reactive glial cells in both conditions. In vitro, we found that proinflammatory stimuli (cytokines and sublytic doses of complement) failed to up-regulate CD55 expression on cultured IMR32 neuronal cells. Interestingly, overexpression of GPI-anchored CD55 on IMR32 was capable of modulating raft-associated protein kinase activities without affecting MAPK activities and neuronal apoptosis. Critically, ectopic expression of decay-accelerating factor conferred strong protection of neurons against complement attack (opsonization and lysis). We conclude that increased CD55 expression by neurons may represent a key protective signaling mechanism mobilized by brain cells to withstand complement activation and to survive within an inflammatory site. The Journal of Immunology, 2005, 174: 2353–2365.

The functions of complement activation include chemotaxis and activation of phagocytic cells, opsonization, phagocytosis, and lysis of target organisms (1, 2). Complement activation occurs sequentially via either the classical pathway involving C1 (C1q, C1r, C1s), C4, C2, C3, or the alternative pathway involving C3, factor B, and factor D. To prevent bystander damage, cells express several key complement regulatory molecules on their plasma membranes. Membrane cofactor protein (MCP, CD46), decay-accelerating factor (DAF; CD55), and complement receptor 1 (CR1; CD35) act at the C3/C5 convertase stage whereas CD59 blocks the assembly of the lytic membrane attack complex (MAC) (3).

There is compelling evidence that sustained activation of the complement system within the CNS causes tissue injury (for recent review, see Refs. 4 and 5). Hence, transudation of plasma through a damaged blood-brain barrier (BBB) can contribute to the deposition of potentially cytotoxic and cytolytic complement components in brain tissue. In addition, local synthesis by brain cells can provide a functional intraparenchymal complement system. Accordingly, complement proteins have been found in the inflamed brain following cerebral ischemia (6, 7) and brain trauma (8, 9). Complement activation products have also been localized within the CNS parenchyma of multiple sclerosis (MS) patients, providing a strong body of evidence that complement-mediated cell death is prominent in areas of active demyelination in multiple sclerosis (10–12). Although the neurotoxic properties of complement are well-established in several inflammatory disorders of the CNS, the intrinsic protective roles of endogenous complement regulatory proteins remain ill-characterized. Expression of complement regulators has been documented in the normal human brain (13) but much less is known about expression in disease conditions. A previous report included preliminary immunohistochemical data.
describing the expression of complement regulators in MS brain tissue sections (11). The authors reported a rather diffuse immunostaining throughout the plaque and the periplaque tissue but did not address the cellular origin of this expression. Additionally, it has been suggested that the low abundance of CD59 on neurons may contribute to neurodegeneration during the course of Alzheimer’s disease (14, 15).

Using experimental autoimmune encephalomyelitis (EAE) in the common marmoset, a model that resembles a chronic MS pathology (16) and EAE in the rhesus monkey, a model that resembles acute disseminated encephalomyelitis (17, 18), we characterized the capacity of neuronal and glial cells to express complement regulatory proteins in response to an inflammatory insult associated with complement activation. Our data show activation of the classical pathway of complement on neurons and axons located in the close vicinity of inflammatory foci. Possibly in response to this insult, a subpopulation of perifocal neurons strongly stained for C3 was found to express high levels of CD55 while expression of other complement regulators was largely unaffected.

These original in vivo data were corroborated in vitro, where we found that several human neuron cell lines expressing low levels of CD55 (IMR32≤<55N, SKNer, LAN1, LASS) were particularly prone to spontaneous complement activation via the classical pathway and prompting robust complement-mediated cell killing. Although the level of MHC class I Ag, the NK innate immune regulator, was strongly up-regulated following neuron cell stimulation with IFN-γ, we found that all neuron human cell lines failed to up-regulate CD55 expression in response to IFN-γ and other proinflammatory stimuli such as sublytic doses of complement (human serum 1:20) and cytokines known to be produced in demyelinating diseases (19). Further in vitro studies of the neuron model cell line indicated for the first time that the GPI-anchored CD55 molecule overexpressed by transfected IMR32 cells was localizing to lipid rafts (also known as glycosphingolipid-enriched membrane microdomains (GEMs)) and, interestingly, promoting raft-associated protein tyrosine kinase activities. Ab-mediated cross-linking of CD55 was found to modulate the phosphorylation of four neuronal proteins, ~85, 95, 110, and 140 kDa, but this treatment failed to induce or modulate MAPK activities and neuronal apoptosis. One of these raft-associated phosphoproteins may correspond to the newly described 80-kDa transmembrane adaptor molecule termed phosphoprotein associated with GEM (PAG) (20). This hypothesis was supported by the detection of high levels of PAG following FACS analyses of five human neuron cell lines. Critically, in vitro experiments revealed that ectopic expression of CD55 on IMR32 conferred robust neuroprotective activity from complement opsonization and MAC-mediated cell lysis.

### Materials and Methods

#### Monkeys

Three marmoset monkeys (*Callithrix jaccus jaccus*, no. M1, M2, and M3) and three rhesus monkeys (*Macaca fascicularis*, nos. R6, R7, and R8) were randomly selected from the purpose-bred colony at the Biomedical Primate Research Center (BPRC; Rijswijk, The Netherlands; Table I). Adjuvant controls included two marmosets (nos. M4 and M5) injected with OVA/CFA and two rhesus monkeys (nos. R9 and R10) immunized with bovine type II collagen/CFA to evoke arthritis. During experiments, monkeys were housed individually in spacious cages with padded shelters provided at the bottom of the cage. The daily diet consisted of food pellets for New World monkeys (Special Diet Services) or for rhesus monkeys, primate food pellets (Hope Farms). The marmosets’ diet was supplemented with rice, peanuts, marshmallows, biscuits, fresh fruits, and vegetables. Drinking water was provided ad libitum. All animal experiments were reviewed and approved by the BPRC’s Animal Care and User Committee.

#### Induction of EAE

EAE was induced in marmosets using *Escherichia coli*-derived recombinant human myelin oligodendrocyte glycoprotein (rhMOG) (21). Marmosets were immunized with 100 µg of rhMOG emulsified in CFA. Under ketamine anesthesia (15 mg/kg; AST farma), each monkey was injected with 600 µl of emulsion into the dorsal skin divided over four locations: two in the inguinal and two in the axillary region. *Borrelia pertussis* was not included in the immunization protocol. Clinical disease course was scored daily by a trained observer using a semiquantitative scoring system: 0 = no clinical signs; 0.5 = apathy, loss of appetite, altered pattern of walking without ataxia; 1 = lethargy and/or anorexia; 2 = ataxia; 2.5 = mono- or paraparesis and/or sensory loss and/or brain stem syndrome; 3 = hemi- or paraplegia; 4 = quadriplegia; 5 = spontaneous death attributable to EAE. For ethical reasons, marmosets were sacrificed when the clinical EAE score of 3 was reached.

EAE was induced in rhesus monkeys by immunization with a synthetic peptide encompassing aa 34–56 of human MOG. The inoculum contained 360 µg of peptide in 500 µl of water emulsified with the same volume of CFA and was injected into the dorsal skin distributed over 10 spots of 100 µl. Monkeys not responding to the initial immunization protocol (no evidence of clinical impairment) received a booster-immunization with MOG peptide in incomplete adjuvant. The detailed immunization protocol in rhesus monkeys was as follows: R6 rhesus monkey: 1 × MOG34 –56/CFA + 1 × MOG34–56/IFA (IFA); R7 rhesus monkey: 1 × MOG34–56/CFA + 2 × MOG34–56/IFA.

#### Table I. Histopathology and expression of complement-related molecules

<table>
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<tr>
<th>Animal Number</th>
<th>Day of Sacrifice</th>
<th>Degree of Demyelination</th>
<th>Infiltration</th>
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<th>Astrocyte Activation</th>
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<td>+ (e)</td>
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<td>+ + + (i, n)</td>
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<td>ND + + (i, c)</td>
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<td>-</td>
<td>-</td>
<td>ND + + (i, c)</td>
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</tbody>
</table>

*a Co, control animals; staining intensity: -, absent; +, weak; + + , moderate; + + + , strong; site of expression: i, infiltrates; e, endothelial cells; m, ramified microglia; n, neurons.*
Neuropathology and immunohistochemistry

Ketamine-anesthetized monkeys were euthanized by an i.v. injection of sodium pentobarbital (Euthesate; APhrno). Brains were snap-frozen in liquid nitrogen and 6 μM serial sections were cut. Some sections were stained with H&E and Luxol fast blue for histopathological examination. Labeling was performed using a protocol based on the immunoperoxidase method (22). Abs used for immunohistochemistry are described in Table II. Briefly, sections were rehydrated before fixation in 4% paraformaldehyde. Endogenous peroxidase activity was quenched by incubation of the sections in 0.3% hydrogen peroxide for 15 min. Cells of the monocytic lineage were detected by acid phosphatase blue staining (23) (see example Fig. 3M) demonstrating endogenous enzyme activity in lyosomes by using naphthol-AS-BI phosphate (Sigma-Aldrich). Sections were incubated with primary Ab overnight at +4°C and then incubated with the appropriate secondary Abs for 1 h at room temperature. Goat anti-rabbit and anti-mouse IgG Abs (1:100) conjugated to HRP were obtained from Bio-Rad. Peroxidase was visualized using 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich) resulting in a red translucent precipitate. Sections were counterstained with hematoxylin and coverslipped with Faramount aqueous mounting medium (DAKO). Double-staining experiments for coexpression of complement regulatory proteins and oligodendrocyte marker CNPase were also performed using the alkaline phosphatase/peroxidase method (22) with goat anti-rabbit IgG Ab (1:100) conjugated to alkaline phosphatase (Sigma-Aldrich). Sections were stained for complement regulatory proteins as described above (AEC), before the final substrate step for alkaline phosphatase activity (CNPase Ab) using naphthol-AS-MX-phosphate and Fast Blue BB base (Sigma-Aldrich). Double immunostainings using either mouse anti-NeuN and rabbit anti-cleaved caspase 3 (see Fig. 1) or rabbit anti-CD55 and goat anti-C3 (see Fig. 3) were performed essentially as described (24). NeuN is a nuclear protein expressed exclusively by neurons (25) and the rabbit anti-caspase 3 recognizes only the cleaved caspase 3 in apoptotic cells. The gliarial intermediate acidic protein (GFAP) staining identified the astrocyte population (26) and microglia/macrophages were stained with the anti-HLA class II Ab (27).

Cell cultures, cytokine stimulation, FACS, and Western blot analysis

Maintenance of cell lines was conducted according to the protocol described previously (24, 28). Human neuroblastoma cell line IMR-32 was obtained from the American Type Culture Collection (ATCC) and cultured in RPMI 1640. SKNER, 55N, LAN1, NMB7, and LA5S neuroblastoma cells were kindly provided by Dr. S. Tomlinson (Department of Microbiology, University of South Carolina, Charleston, SC) (29). The cells were obtained from PeproTech and used at 10 ng/ml over a period of 24 h. FACS analysis was conducted as described (31) using several mAbs from IBGRL (anti-CD59, clone BRIC 229; anti-CD55 clone BRIC216; anti-CD47, clone BRIC126) and Dr. V. Horejsi (Institute of Molecular Genetics, Prague, Czech Republic) (anti-PAG clones MEM252–255; anti-CD56, clone MEM188). Abs against Thy-1 (clone K117) and HLA class I Ag (clone W6/32) were isolated from tissue culture supernatants (TCS) of hybridoma cell lines (ATCC). CD56 and Thy-1 Abs are GPI-anchored molecules defining the neuronal phenotype of the cell lines and can be used as tracers of neuronal lipid rafts.

Western blotting was performed on cells solubilized in PBS containing 2% Nonidet P-40 together with protease inhibitors as previously described (31). Cell lysates were fractionated on 10% SDS-PAGE, electroblotted onto nitrocellulose and stained overnight at 4°C with rabbit anti-CD55 (1:1000) or rabbit anti-CD46 produced in our laboratory. After washing and incubation with peroxidase-labeled goat anti-rabbit IgG (1:1000; Bio-Rad), blots were developed using the ECL system (ECL; Pierce). Prestained broad range protein markers from New England Biolabs were used as m.w. standards.

Assays to measure complement-mediated opsonization and cell lysis

C3 deposition was assessed as described previously with slight modifications (32). Cells were harvested by incubation in PBS/10 mM EDTA. Cells (105 cells/ml) were washed to remove the chelating agent and then incubated for 30 min at 37°C with dilutions of C8-depleted human serum. Negative controls comprised incubation of cells with C8-depleted serum in the presence of 10 mM EDTA. Cell viability was monitored by trypan blue exclusion. Cells were washed twice in FACS buffer (PBS/1% BSA) and stained for C3 using Abs against C3b (monoclonal mouse anti-human C3b neoeptipe, clone C3/30; TCS 1:100); (33), C1q (polyclonal rabbit anti-human C3d; DAKO; 1:50), and C3d (polyclonal rabbit anti-human C3d; DAKO; 1:500), followed by PE (R-PE)-conjugated donkey anti-mouse or anti-rabbit Ig (Jackson ImmunoResearch Laboratories; 1:200). Complement deposition was measured using flow cytometry.

Cell lysis was quantified by measuring the release of the fluorescent marker calcine (28). To this end, 5 × 104 cells were incubated at 37°C for 30 min with 200 μl of calcine-AM (Molecular Probes) at 2 μg/ml in culture medium. Cells were carefully rinsed once in medium and incubated at 37°C for 1 h with 200 μl of a dilution of either normal human serum or

<table>
<thead>
<tr>
<th>Table II. Conditions, sources, and manufacturers of Abs used*</th>
<th>Ab (Ag)</th>
<th>Species</th>
<th>Dilution</th>
<th>Source</th>
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<tr>
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* As, ascites.
** Concentration of IgG cut following purification on protein-A sepharose.

Table II. Conditions, sources, and manufacturers of Abs used*
heat-inactivated human serum as a negative control. In some experiments, complement-mediated lysis was enhanced by coincubation with the neutralizing Ab to CD59 (BRIC229; 10 μg/ml) as described previously (28). Plates were chilled on ice and supernatant was transferred to a flat-bottom 96-well plate for analysis using a Fluorescence plate reader (Victor, EG/G Wallac). Calcein remaining in cells was released by incubation for 15 min with 200 μl of 0.1% Triton X-100 in PBS.

Ectopic expression of human CD55 cDNA in IMR-32 cells

Human CD55 cDNA cloned in the expression vector pDR2ΔEF1a carrying the hygromycin resistance gene was a gift from Dr. I. Anegon (Institut National de la Santé et de la Recherche Médicale (INSERM Unité 437) and has been described previously (34). IMR-32 cells were transfected using lipofectamine (Invitrogen Life Technologies) with the CD55-containing vector or with the empty expression vector (negative control) and stable cell lines were generated essentially as described (35). CD55 expression was confirmed using immunocytochemistry, FACS analyses, and Western blotting. CD55 is known to be anchored in the outer membrane leaflet via a GPI anchor and may signal through adaptor molecules yet to be identified (36–38). Hence, several experiments were conducted to characterize the plasma membrane organization and the cell signaling properties of rCD55 expressed by IMR32 cells.

First, transfected cells were double-stained for CD55 using the polyclonal Ab and for control GPI-anchored molecules (CD56 and Thy-1, using mAbs). CD55-transfected IMR32 and empty vector-transfected IMR32 were cultured on glass coverslips coated with poly-β-lysine (10 μg/ml for 30 min). Coverslips were thoroughly washed five times in PBS and fixed in 4% formaldehyde for 10 min. Coverslips were immersed for 30 min in PBS/BSA to block the nonspecific Ab binding. Coverslips were then incubated with primary Abs in 200 μl of PBS/BSA at 4°C overnight in a humidity chamber. After washing, coverslips were incubated for 1 h at room temperature with TRITC-labeled donkey anti-rabbit IgG and FITC-labeled donkey anti-mouse FITC (1:100; Jackson ImmunoResearch Laboratories). After intensive washing, coverslips for fluorescence microscopy were mounted in Fluoromount (DAKO). Codistribution of the fluorescence signals were imaged using a fluorescent microscope (Leica DMLB).

Second, the association of CD55 in lipid rafts was tested using the sucrose gradient protocol described by Briddica et al. (20). Briefly, 2 × 10⁶ cells were lysed in 250 μl of 1% Nonidet P-40 (NP40; Calbiochem) at 4°C for 30 min. Cell extract was placed at the bottom of ultracentrifugation tubes, made 40% sucrose (w/v) in a 0.5-ml volume and overlaid with 35% sucrose 1.75 ml followed by 250 μl of 1% NP40. Ultraconcentrifugation was performed at 250,000 × g for 18 h at 4°C and eight fractions were collected from the top of the gradient. Brief sonication on ice of the remaining nuclear pellet constituted the fraction number 9. Protein present in lipid raft fractions (top of the gradient) were detected by Western blotting.

The functional role of CD55 present in lipid rafts was tested using three independent assays: 1) robust tyrosine phosphorylation assay essentially as described (20); 2) measuring at the level of p44/p42, p38 MAPK activation by FACS analysis; and 3) examining whether DAF signaling controls neuron apoptosis. For the tyrosine phosphorylation assay, IMR32 control cells (expressing low levels of endogenous CD55) or CD55 plasmid transfected IMR32 were treated with monoclonal anti-CD55 Abs (BRIC 216 and BRIC 110, at 10 μg/ml for 2 × 10⁶ cells) for 10 min at 4°C. Ab-coated cells were placed at 37°C for 10 min either alone or with 10 μg/ml of a rabbit or goat anti-mouse (GAM) Ab (F(ab’))₂; Sigma-Aldrich) to further induce cross-linking. Cells were lysed for 30 min in 1% NP40 buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM MgCl₂, 1 mM EGTA) in the presence of protease inhibitors and phosphatase inhibitors (50 mM NaF, 1 mM Na₃VO₄). Samples were centrifuged (13,000 × g, 10 min) and postnuclear supernatants were subjected to SDS-PAGE and anti-phosphotyrosine immunoblotting using P-TYR01 and P-TYR02 mAbs (kindly provided by Dr. V. Horejsi). In some experiments, cells were exposed to pervanadate (5 mM for 10 min) as a control for cell activation and tyrosine phosphorylation. For MAPK activation assays, DAF was cross-linked as described above using the mouse anti-DAF + GAM and cells were permeabilized in cold methanol before immunostaining with either rabbit anti-phospho-p44/p42 or rabbit anti-phospho-p38 MAPK (Cell Signaling Technology). The level of MAPK phosphorylation was analyzed by FACS. Finally, for the apoptosis assay, cells starved overnight in medium without FCS were incubated for 6 h with either different mouse anti-DAF Abs (BRIC216, BRIC230, BRIC128, BRIC156, IBGRL) or mouse anti-GPI-anchored prion protein (PRION308 clone, 6G1; SFIBIO) or mouse anti-CD55 mAb (BRIC156) for 10 min at 4°C. Plasminogen activator inhibitor type 1 (PAI-1) activity, acting as a negative control, was assayed with a specific chromogenic substrate (S-2251, Bachem) incubated at 37°C for 10 min, the reaction was stopped with 30 μl of 10% trichloroacetic acid. Acetylated plasminogen was added to scavenge any uncleaved substrate, and the residual activity was determined spectrophotometrically at 405 nm.

Results

EAE in monkeys is characterized by numerous perivascular inflammatory infiltrates, demyelination, and marked glial activation

The disease duration differed among outbred animals confirming previous findings (Table I) (16, 39). The degree of demyelination and the presence of inflammatory infiltrates were assessed by Luxol fast blue and H&E staining (Table I). The data regarding the astroglial activation (GFAP staining) and microglial activation (HLA class II staining) are summarized in Table I. EAE in the marmoset follows a relapsing-remitting or chronic progressive course associated with active demyelination (40, 41). Lesions were characterized by reduced density of myelinated fibers as assessed by Luxol fast blue staining (Fig. 1A). In marmoset EAE brains, staining for HLA DP, DQ, DR indicated a high number of perivascular infiltrating macrophages localized around blood vessels within the lesions as well as ramified and ameboid microglia/macrophages within active plaques (Fig. 1B). Rhesus monkeys are highly susceptible to EAE induced by MOG34−56 (17). The hyperacute course of the disease observed in the CNS of most MOG peptide-immunized rhesus monkeys contrasts with the more chronic form of EAE described in the marmoset. Accordingly, EAE in the rhesus monkey, in its clinical and pathological presentation, has been proposed to more closely resemble postinfectiousencephalomyelitis than chronic MS (18). Histopathological evaluation of rhesus EAE brains showed multiple foci of infiltration and necrotic demyelinating lesions with severe inflammatory infiltrates in both gray (Fig. 1C) and white matter (data not shown). Foci of inflammatory infiltrates in rhesus EAE contained numerous macrophages positive for HLA DP, DQ, DR (Fig. 1D). Lesions in marmoset EAE brains were characterized by active demyelination and oligodendrocyte cell loss as evidenced by the loss of staining for CNPase, a marker for oligodendrocytes (Fig. 1E). These lesions were infiltrated by macrophages containing intracytoplasmic vesicles of myelin debris that were reactive for CNPase (Fig. 1F). Immunostaining using rabbit anti-GFAP Ab showed numerous reactive astrocytes surrounding foci of inflammatory infiltrates in marmoset EAE brains (Fig. 1G). In rhesus EAE brains, not only perifocal reactive astrocytes, but also frequent astrocytic processes within the infiltrates were observed (Fig. 1H). The rabbit anti-cleaved caspase 3 Ab was used to ascertain the overall level of apoptosis in neurons double-stained using the mouse anti-neuronal nuclei (NeuN). In marmoset EAE, very few NeuN-positive cells were stained for caspase 3 (Fig. 1J) and this was in sharp contrast to rhesus EAE brains displaying strong levels of neuronal apoptosis (Fig. 1L).

Robust activation of the classical pathway of complement in EAE lesions

The presence of complement proteins in monkey EAE brains was assessed using immunohistochemistry. Levels of immunoreactivity for complement opsonins (C1q and C3b) are summarized in Table I. Importantly, no complement activation was detected in control brain tissue sections (Table I). In sharp contrast, strong C1q immunostaining was detected next to inflammatory infiltrates and BBB in both EAE models (Fig. 2, A and B). C3b immunoreactivity as detected by a monoclonal anti-neoepitope in marmoset mono-
inflammatory infiltrates exhibited a diffuse pattern spreading into the parenchyma (Fig. 2C). C3b immunoreactivity in rhesus infiltrates appeared to be restricted consistently to the subendothelial Virchow-Robin spaces (Fig. 2D). Parenchymal reaction was characterized by C1q staining on a subset of ramified microglial-like cells in areas surrounding inflammatory infiltrates in marmoset (Fig. 2E) and rhesus (Fig. 2F). C1q and C3b immunoreactivities were never found to be associated with oligodendrocytes in either model perhaps as a consequence of marked loss of oligodendrocytes in areas of demyelination (data not shown).

The complement cascade is activated on axons and perifocal neurons

In the marmoset, C1q-positive axons and neuronal cell bodies were observed in the gray matter bordering infiltrating sites (Fig. 2G) whereas gray matter distant from lesion sites (Fig. 2G, inset) or in control brains (data not shown) was negative for C1q. This observation confirms the key role of the classical pathway in complement-mediated opsonization. In addition, C3b immunoreactivity was present on neurons in marmoset EAE brains (Fig. 2H). Examination at high power consistently showed that C3b was located at the cell membrane of intact neurons (Fig. 2I). Moreover, neurons located in the close vicinity of vessels were found to be strongly immunoreactive for C3b (Fig. 2J).

Moreover, in rhesus EAE, C1q immunoreactivity was present on perifocal neurons but not on axonal extensions (Fig. 2K). The granular pattern of neuronal C1q immunoreactivity was cytoplasmic rather than membrane-associated in contrast to what was observed in the marmoset. Although no infiltrates were present in the R7 rhesus EAE brain (see Table I), neurons were strongly stained for C1q (Fig. 2L). C3b deposition on neurons was never observed in rhesus EAE brains (data not shown). The sheep anti-C9 Ab displayed a characteristic granular/vesicular staining on neuronal bodies (Fig. 2, M and N) and axons (Fig. 2O, arrowheads) in marmoset EAE brains. Weak C9 staining at background level was detected in control and rhesus EAE brains (data not shown).

Complement regulatory proteins are upregulated in EAE lesions

Immunohistochemistry was performed to assess whether membrane-bound complement regulatory proteins were differentially expressed in the brain during the course of EAE. To this end, we used polyclonal and mAbs raised against CD35, CD46, CD59, and CD55. These Abs have been characterized and previously validated in detail (13). A summary of the staining profiles is presented in Table I which shows that several complement regulatory proteins were upregulated in response to EAE pathology. CD35 immunoreactivity was not detected in the brain parenchyma of the control animals (Fig. 3B) but was found on neutrophils in vessels (Fig. 3A). Infiltrating inflammatory cells in marmoset (Fig. 3C) and rhesus (data not shown) EAE brains were weakly stained for CD35. CD35 staining was negative or below detectable levels in the parenchyma of both EAE models. Expression of CD59 in control brains was restricted to endothelial cells (Fig. 3D). In marmoset EAE, weak and diffuse CD59 expression was detected close to the inflammatory infiltrates (Fig. 3E). Unfortunately, polyclonal and monoclonal Abs raised against human CD59 did not cross-react with rhesus CD59. CD46 immunoreactivity in control brains was found solely on endothelial cells (Fig. 3F). Remarkably, foci of infiltration were found to be strongly stained for CD46 in both marmoset (Fig. 3G) and rhesus EAE (Fig. 3H). CD46-labeled microglial cells were found in white matter tracts of R7 and R8 rhesus EAE brains (Fig. 3I). In control brains, CD55 immunoreactivity was present on vessels (Fig. 3J) but was not detected in white
matter parenchyma of control marmoset brains (Fig. 3K). Inflam-
matory infiltrates were found to be positive for CD55 in marmoset (Fig. 3L) and rhesus (Fig. 3M) EAE brains. Surprisingly, double
immunohistochemistry staining using cell-specific marker Abs re-
vealed that the expression of complement regulatory proteins on
astrocytes and on spared oligodendrocytes was below detectable
levels (data not shown).

**FIGURE 2.** Immunostaining for complement opsonins (C1q and C3b) and C9 in marmoset and rhesus EAE brains. Control brain tissue sections failed
to display C1q, C3b (G and H, insets) and C9 stainings (not shown). In contrast, strong C1q staining was associated with perifocal inflammatory infiltrates
in marmoset (A) and rhesus (B) EAE brains (polyclonal anti-human C1q). A strong staining for C3b (C/30 mAb) is detected on reactive endothelia and
close to the cell infiltrates in marmoset (C) and rhesus (D) EAE brains. Note: In rhesus EAE brains, C3b immunoreactivity was restricted to the close vicinity
of infiltration sites. The basal lamina was frequently stained for C3b (D, arrows). C1q-immunopositive ramified microglial cells located at the edge of the
lesion were observed in both marmoset (E, arrows) and rhesus (F, arrows) inflamed brains. Not all microglial-like cells were found to be positive for C1q
(F, arrowheads). G, Perifocal neuron bodies and axon-like structures were strongly stained for C1q in marmoset EAE. No staining for C1q was observed
in gray matter of control marmoset (G, inset). H, C3b opsonization was also depicted on neurons located in the close vicinity of inflammatory infiltrates
in marmoset EAE brains and, in particular, localizing to the cell surface (I, arrows). J, C3b deposition on neurons (arrowhead) next to brain blood vessels
(arrow) was frequently observed. In rhesus EAE brains, C1q-immunopositive neurons are in areas surrounding the lesions (K), whereas control rhesus gray
matter is negative for C1q (K, inset). L, R7 rhesus monkey, displaying C1q staining of neuron-like bodies in the gray matter. A characteristic granular/
vesicular C9 staining was detected on neuronal bodies and axonal structures in marmoset EAE brains. Arrowheads in panel O point to three distinct

**Chronic activation of the complement cascade in EAE brains promotes overexpression of CD55 by a subset of neurons**

Neurons in control and EAE cases did not display detectable levels
of CD35, CD46, and CD59 (data not shown). Moreover, neurons
failed to express CD55 in control brains. In sharp contrast, in all
marmoset EAE cases, CD55 expression was found to be induced de
novo on neurons surrounding sites of inflammatory cell infiltration.
FIGURE 3. Complement regulatory proteins (CD35, CD59, CD46, and CD55) are differentially expressed and regulated in EAE pathology. A, In control marmoset brain, we found rare polynucleated cells close to brain blood vessels strongly stained for CD35 (arrowheads). CD35 was neither detected on neurons (gray matter, B) nor on glial cells in the white matter. The CD35 staining of normal rhesus brain sections confirmed these findings. C shows that only a few perivascular inflammatory cells were stained for CD35 in marmoset EAE brain. D, mAb to human CD59 stained brain blood vessels in control marmoset. E, Diffuse CD59 staining was also present in inflammatory foci in marmoset EAE brain. F, The polyclonal Ab to human CD46 stained brain blood vessels in control marmoset but not neurons and glial cells. Perivascular inflammatory infiltrates were strongly stained for CD46 in marmoset (G) and rhesus (H) inflamed brains. I, CD46-positive ramified microglia in the vicinity of a lesion in rhesus EAE brain. J, CD55 immunoreactivity was observed on endothelial cells of brain blood vessels (arrows) and on circulating cells (arrowheads) in control rhesus brain. Gray matter cells in control marmoset brain were totally negative for CD55 (K). CD55 is present in inflammatory infiltrates in marmoset (L) and rhesus (M) EAE brains. Note: endogenous lysosomal acid phosphatase activity is detected in cells of the monocytic lineage using naphthol AS-BI phosphate (M, blue staining, arrows). In contrast to control brains, we found that neurons surrounding site of inflammatory cell infiltration (∗) were strongly stained for CD55 (arrowheads) in marmoset EAE brains but not in rhesus EAE (data not shown). O shows a higher magnification of perifocal neurons immunopositive for CD55. CD55 immunoreactivity on neurons decreases with distance from the lesion (P). Neuronal bodies and axons strongly stained for C3 (goat anti-C3) were double-stained for CD55 in marmoset EAE (Q and R). In contrast, no C3b and CD55 staining was detected on neurons in rhesus EAE brains. We confirmed that the polyclonal anti-CD55 stained erythrocytes and smooth muscle cells in rhesus while the goat anti-C3 stained the lumen of vessels. Original magnification: N, ×100; C, E, G, H, K–M, O, P, ×250; A, B, D, F, J, Q–T, ×400; I, ×800.
that IMR32 expressed receptors for all three cytokines (data not shown). Neurons exposed to acute inflammatory insults in rhesus EAE brains failed to up-regulate CD55 expression (Table I). We confirmed that both Abs cross-reacted against rhesus proteins with the staining of erythrocytes and smooth muscle cells for CD55, while the anti-C3 Ab strongly stained the lumen of vessels (Fig. 3, S and T).

Cultured neurons expressing low levels of CD55 are highly susceptible to complement-mediated lysis

Because our in vivo EAE data clearly indicated that some neurons are subject to complement attack but survive by overexpressing CD55 we decided to further explore the key role of CD55 in signaling neuroprotection in the context of a chronic innate immune response.

First, we screened seven human neuron cell lines for expression of complement regulators by FACS analysis and Western blotting of cell lysates (Fig. 4, A and B). In agreement with published data on human fetal neurons (13, 42), we found that all neuron cell lines failed to express CD35 (data not shown), had low levels of CD55 while CD46 and CD59 levels were comparable to the levels expressed on several control leukocyte cell lines (THP1, YT, K562). Interestingly, IMR32 neuron cells expressing low levels of CD55 (Fig. 4, A and B) spontaneously activated the classical pathway of complement and consequently prompted strong C1q opsonization (data not shown) and complement-mediated cell lysis (Fig. 4C). Encouraged by the IMR32 data which are reminiscent of the original EAE observations, we asked whether the expression of CD55 on neurons could be modulated by proinflammatory cytokines known to be expressed in inflammatory brain conditions.

Proinflammatory cytokines and sublytic doses of complement fail to induce CD55 overexpression on IMR32 cells

There is compelling evidence that proinflammatory cytokines as well as complement opsonins themselves promote the expression of complement regulators for example on endothelial cells (HUVEC) helping to maintain vascular integrity during inflammatory insults (43). When IMR32 cells were exposed to either recombinant IFN-γ, IL1-IL11 TNF-α or sublytic doses of complement (NHS 1:20) for 24 h, we found no significant differences in CD59, CD46, and CD55 levels between treated cells and control cells (Fig. 5 and data not shown). CD35 was not expressed by IMR32 following cytokine stimulation. RT-PCR data confirmed that IMR32 expressed receptors for all three cytokines (data not shown), and furthermore, it was clear that IFN-γ was able to induce strong expression of MHC class I Ag on IMR32 cells a regulatory protein to protect from innate immune NK cell killing (Fig. 5A). CD59 but not CD55 expression was up-regulated when IMR32 cells were treated with PMA to induce cell differentiation.

GPI-anchored CD55 in lipid rafts promotes neuron cell signaling without stimulating MAPKs and programmed cell death

We used the IMR32 model cell line to test for the signaling properties of GPI-anchored CD55. First, we found that CD55 molecule was localizing to lipid rafts in CD55-transfected IMR32 cells and two other human neuron cell lines (Fig. 6B). Lipid rafts fractions were identified by the Thy-1 and CD56 immunoblots, floating on the top of the sucrose gradient as expected (data not shown). Furthermore, cross-linking of CD55 was found to modulate tyrosine phosphorylation of four neuronal proteins, ~85, 95, 110, and 140 kDa (Fig. 6C). One of these raft-associated phosphoproteins may correspond to the newly described 80-kDa transmembrane adaptor molecule termed PAG (20). This hypothesis was supported by the detection of high levels of PAG following FACS analyses of five human neuron cell lines (Fig. 5A). The treatment of IMR32 and IMR32-CD55 with anti-DAF Abs did not affect the level of phosphorylation of p44/42 and p38 MAPKs (data not shown). IMR32 and IMR32-CD55 displayed similar levels of apoptosis in response to camptothecin (Fig. 7A), an inhibitor of topoisomerase I, and the level of cleaved PARP and cleaved caspase 3 was not affected in response to CD55 cross-linking (Fig. 7B).

Ectopic expression of CD55 increased protection from complement attack, opsonization, and cell lysis

Incubation of vector control-transfected IMR-32 cells with dilutions of C8-depleted human serum led to a dose-dependent accumulation of opsonins C3b (Fig. 8Aa), iC3b (Fig. 8Ab), and C3d (Fig. 8Ac). No cell lysis was observed in these experimental conditions (data not shown). No opsonization of cells was observed upon incubation with C8-depleted serum in the presence of EDTA. Overexpression of CD55 was found to markedly inhibit complement deposition on IMR-32 cells (Fig. 8A).

Protection from complement lysis by CD55 expressed on IMR-32 cells was evaluated in a calcein release assay. Incubation of IMR-32 cells transfected with vector alone with normal human serum at a dilution of 1/2 provoked the killing of ~40% of neurons over 4 h (Fig. 8Bb). Expression of CD55 significantly protected IMR-32 cells against complement-mediated lysis (Fig. 8B). Addition of the neutralizing anti-C59 Ab in the bathing medium caused enhancement of cell killing (Fig. 8B). In these conditions, the neuroprotective properties of expressed CD55 were still observable (Fig. 8B).

Discussion

The first outcome of our study is to support the classical wisdom that complement activation in the brain promotes pathology, a consequence of the extraordinary susceptibility of neurons and oligodendrocytes to complement-mediated attack (for review, Ref. 44). In acute brain inflammation, complement activation triggers formation of the MAC that binds to neuronal and axonal membranes promoting neuronal impairment. C1q and C3b opsonins will promote strong phagocytosis of necrotic cells and damaged myelin. Complement C3a/C5a anaphylatoxins released in the fluid phase will drive chemotaxis and recruitment of macrophages and T cells to the brain (24, 45).

The rhesus acute EAE model displayed white and gray matter pathological hallmarks. In the white matter, and in line with previous findings in MS brains, we found abundant immunoreactivity for complement opsonins C1q and C3b within parenchyma and around perivascular infiltrates (10–12, 46). In rhesus EAE, there was a clear tendency for complement to remain associated within the perivascular spaces and close to the basal lamina (Fig. 2, B and D). Interestingly, a subset of ramified but not ameboid microglial-like cells was found to be strongly positive for C1q (Fig. 2F). This observation is consistent with previous studies that identified microglia/macrophages as a major source of C1q in the inflamed brain (7, 47). Deposition of complement activation products such as C3d or C9 neoantigen on myelin sheath has been described in several studies (11, 12, 46, 48). In contrast to these findings in human MS, we were unable to detect C1q and C3b on the myelin sheath itself or on myelin debris in rhesus EAE. This apparent
The surface expression of complement regulatory proteins (CD59, CD55, CD46) and neuronal-specific markers (GPI-anchored CD56 and CD47) was assessed by flow cytometry using mAbs and recombinant PE-conjugated secondary Ab (all listed in Materials and Methods). Data are presented as mean of FL2 of three experiments on separate cultures. (A, a–f), cells were incubated with C8-depleted normal human serum (NHS, 1/10 for 1 h at 37°C) and the level of spontaneous C3b opsonization (as an indication of complement activation) was measured by FACS using the anti-C3b neoepitope (clone C3/30) Ab. B, The level of CD55 and CD46 in neurons (SKNER, PAJU, 55N, NMB7, NT2, IMR32, LAN1) and control (THP1, human fibroblast, YT NK and K562 erythroleukemia) cell lysates was assessed by Western blot using polyclonal affinity purified Abs. C, To ascertain the susceptibility of the neuron cell lines to complement-mediated attack, we used the calcein-release assay to measure the percentage of cell lysis following exposure to NHS without or with the neutralizing anti-CD59 Ab (clone BRIC 229). Remarkably, IMR32 cells which expressed low levels of CD55 were particularly susceptible to complement attack.
discrepancy between this model and MS might simply be explained by the severity of the inflammatory insult and the extensive myelin loss observed at sites of inflammatory cell infiltration in the EAE brain tissues. In the gray matter, the EAE pathology in the rhesus model resulted in a profound neuronal loss associated with robust apoptosis (Fig. 1L). Immunohistochemistry was conducted to test for the expression of complement regulatory proteins. As expected, immunostaining for all membrane-bound complement regulators was associated with inflammatory cell infiltrates (CD46/H11022, CD55/H11022, CD59/H11022, CD35). Interestingly, small clusters of ramified microglial cells expressed high levels of CD46, a key regulator of the C3b amplification loop. Surprisingly, and despite the strong level of astrogliosis in rhesus EAE, no prominent staining for complement regulators was found on astrocytes. Similarly, the few spared oligodendrocytes at the edge of the lesions remained negative for complement regulators, as assessed by double staining with the anti-CNPase Ab. Very little is known about the expression of complement regulatory proteins by oligodendrocytes but previous in vitro experiments as well as immunostaining of human brains suggested that oligodendrocytes can at least express low levels of CD59 (49). In rhesus EAE, we found that neurons expressed no detectable levels of CD59, CD46, CD35, and CD55 suggesting that they would be subject to a full complement attack leading to the observed neuronal loss (17).

The classical role of complement in demyelination, axonal loss and neuronal impairment was also evidenced from the data obtained in the marmoset EAE model. Complement opsonins were localized to the BBB, axons, and neuronal membranes. However, due to the chronic nature of this model mainly affecting the white matter with little impact on the gray matter (the neuronal niche), we were able to address whether complement attack could also translate into neuroprotective mechanisms. Remarkably, our immunohistochemistry data show for the first time that deposition of C3 opsonin at the neuronal cell membrane is accompanied by de novo expression of CD55, suggesting a causal link between opsonization and the capacity of cells to respond by expressing increased levels of complement regulators (Fig. 3, N, O, Q, and R). This interesting paradigm bears some resemblance to the observation that exposure of cultured HUVEC to sublytic doses of complement stimulates complement regulator expression (43). We found small clusters of C9 stainings on healthy-looking neurons in marmoset EAE and it is possible that such sublytic doses of the C5b9 complex signal neuroprotective activities and have antiapoptotic effects as described for other CNS cells (50). Experiments to further test this hypothesis are highly warranted.

Recent microarray data have indicated that the level of CD55 mRNA is up-regulated in brain disease conditions but without addressing the physiopathological relevance of this finding (51). We made use of several human neuron cell lines to decipher the signaling and neuroprotective roles of CD55 on neurons. First, we confirmed that CD55 is the most important complement regulator...
on neurons because cells with low CD55 (IMR32) displayed strong complement-mediated lysis (Fig. 4).

There is a strong body of evidence that exposure of neuronal cells to proinflammatory cytokines results in marked phenotypic changes including the up-regulation of adhesion molecules and the secretion of soluble mediators (e.g., growth factors) (for review, Ref. 19). Surprisingly, proinflammatory cytokines and sublytic doses of complement failed to increase CD59, CD46, or CD55 expression on five neuron cell lines and these findings are in sharp contrast to the data reported on endothelial cells (43). Interestingly, some of these treatments induced robust expression of the MHC class I Ag, the NK innate immune regulator, by a factor of 10- to 20-fold (Fig. 5). It remains to be tested whether exposure of neurons to C3a or C5a anaphylatoxins can up-regulate the expression of complement regulators.

Additional experiments were performed to ascertain the emerging roles of the nonclassical complement receptors (CD59, CD46, and CD55) in promoting neuronal cell signaling. CD55 is a GPI-anchored glycoprotein, which, because of its method of cell surface anchoring, does not span the plasma membrane (37). Despite this, we here report for the first time that Ab-mediated cross-linking of CD55 on IMR32 resulted in a rapid and robust increase in the level of tyrosine phosphorylation of several intracellular proteins (Fig. 6). It will be interesting to ascertain whether C3b, the CD55 natural ligand, is capable of similar activities. One of these phosphoproteins could be the PAG adaptor molecule key to the...
control of T cell activation (Fig. 5). Coimmunoprecipitation experiments will help to test this hypothesis. Interestingly, PAG is known to bind to the tyrosine kinase Csk, the major negative regulator of Src kinases (20). The cellular events promoted by CD55 signaling are unknown and differential display experiments will help to delineate the expression of novel cellular markers induced by CD55 signaling. The neuroprotective effects of overexpressed CD55 on neurons were remarkable in the face of strong complement opsonization and even when CD59 was neutralized using a blocking Ab (Fig. 8). It is clear that CD55 overexpression represents an important mechanism to protect neurons against complement-mediated damage. This paradigm corroborates the observations of Zhang and colleagues (30) describing that PMA-induced overexpression of CD55 on the PAJU neuronal cell line conferred elevated resistance to complement-mediated lysis. Using our model, it will be important to investigate the roles CD55 and other GPI-anchored proteins have in regulating cell growth, proliferation, and how this may be altered in diseases. For example, it has been hypothesized that the absence of CD59 on neurons in Alzheimer’s disease could be involved either in MAC-mediated neuronal loss or in failure to signal neuroprotective activities (15).

In conclusion, one important finding that has emerged from this report is that a subpopulation of neurons express protective molecules to maintain neuronal integrity in the face of an innate/adaptive immune challenge.

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References


13. Yang, L. B., R. Li, S. Meri, J. Rogers, and Y. Shen. 1999. Inhibition of oligoden-


27. Yasuhara, B., B. R. Little, S. J. Smiley, J. Rogers, and Y. Shen. 2000. Deficiency of complement defense protein CD59 may contribute to neurodegeneration in Alzhe-


29. t Hart, B. A., M. van Meurs, H. P. Brok, L. Massacesi, J. Bauer, L. Boon, R. E. Bouton, and J. D. Laman. 2000. A new model for multiple sce-


32. myelitis induced by myelin oligodendrocyte glycoprotein: characterization of imm-

33. unilaterial experimental autoimmune encephalomyelitis in the common mar-

34. moset (Callithrix jacchus) Prevention of experimental autoimmune encephalomyelitis in the common marmoset (Callithrix jacchus) using a chimeric antigen monoclonal antibody against human CD40 is associated with altered B cell responses. J. Immunol. 167:2942.


42. moset (Callithrix jacchus) using a chimeric antigen monoclonal antibody against human CD40 is associated with altered B cell responses. J. Immunol. 167:2942.


49. Soane, L., H. Rus, F. Niculescu, and M. L. Shin. 1999. Inhibition of oligoden-
