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Neuronal Expression of a Functional Receptor for the C5a Complement Activation Fragment

Stephen A. O’Barr, Jody Caguioa, Donna Gruol, Guy Perkins, Julia A. Ember, Tony Hugli, and Neil R. Cooper

The present studies were undertaken to determine whether neuronal subsets in normal brains constitutively express functionally competent C5a receptors. In situ hybridization studies coupled with immunohistochemical approaches revealed that most neurons in the hippocampal formation, many pyramidal cortical neurons, and cerebellar Purkinje neurons in normal human and murine brains constitutively express C5a receptors. Neuronal C5a receptors bound C5a-coated fluorescent microspheres, and primary rodent hippocampal neurons responded to C5a with increased calcium fluxes via a pertussis-sensitive, presumably Gi-coupled protein. Additional studies with human neuroblastoma cells conducted to address the functional role of C5a receptors revealed that C5a triggered rapid activation of protein kinase C and activation and nuclear translocation of the NF-κB transcription factor. In addition, C5a was found to be mitogenic for undifferentiated human neuroblastoma cells, a novel action for the C5aR. In contrast, C5a protected terminally differentiated human neuroblastoma cells from toxicity mediated by the amyloid Aβ peptide. Thus, normal rodent hippocampal neurons as well as undifferentiated and differentiated human neuroblastoma cells express functional C5a receptors. These results have implications for understanding the role of neuronal C5aR receptors in normal neuronal development, neuronal homeostasis, and neuroinflammatory conditions such as Alzheimer’s disease. The Journal of Immunology, 2001, 166: 4154–4162.

Complement 5a, a 74-residue proteolytic activation fragment of the fifth component of the complement system (C5), has long been known to play an important role in host defense by acting as a chemotaxin and activator of myeloid cells (for reviews, see Refs. 1 and 2). In addition, C5a augments inflammatory responses by triggering the production and release of proinflammatory cytokines, bioactive amines, superoxide anion, and intracellular enzymes from cells of myeloid origin. Localized injection of C5a increases vascular permeability, C5a is a member of the superfamily. Although capable of stimulating intracellular signaling pathways leading to chemotactic responses in a variety of cell types, the C5aR is not a member of any of the Gi5 subunit families. Ligation of the C5aR on various cell types leads to activation of phospholipase C, phosphatidylinositol-3-kinase, and the ras/raf/mitogen-activated protein kinase pathway (2, 6).

Thus, normal rodent hippocampal neurons as well as undifferentiated and differentiated human neuroblastoma cells express functional C5a receptors. These results have implications for understanding the role of neuronal C5aR receptors in normal neuronal development, neuronal homeostasis, and neuroinflammatory conditions such as Alzheimer’s disease. The Journal of Immunology, 2001, 166: 4154–4162.

The C5aR was initially characterized on myeloid cells and thought to be confined to cells of this lineage; however, more recent studies have shown that numerous nonmyeloid cell types, including endothelial, epithelial, and other cells in skin, liver, lung, heart, kidney, and intestine express the C5aR (7–10). Constitutive expression of the C5aR on nonmyeloid cells suggests as yet undetermined noninflammatory roles for C5a.

Within the CNS, several groups have demonstrated that astrocytes and microglia constitutively express the C5aR, both in vitro (11–13) and in vivo (14), and that expression of the receptor is increased in diverse inflammatory conditions (10, 14). The glial C5aRs are functionally competent since C5a stimulates calcium fluxes, induces cytokine transcription, triggers rearrangement of the cytoskeleton, and stimulates chemotactic activity of the cells (11, 15–18).

Surprisingly, subsets of neurons in various brain regions of mice experiencing diverse inflammatory conditions have been found to express the same C5aR as found on peripheral myeloid cells, astrocytes, and microglia. This has been demonstrated at the mRNA and protein levels for neurons in vivo in transgenic (tg) mice expressing IL-3 in astrocytes (19) and in other inflammatory models in mice including bacterial meningitis (20), postintraarticular injection of TNF-α (20), traumatic injury (21), and postventricular injection of kainic acid (22). The neuronal C5aR in kainic acid-injected mice has been shown to bind radiolabeled recombinant C5a (22).

C5aR expression was clearly induced by the inflammatory stimuli in these studies, since neuronal subsets in the brains of normal mice have been reported to express essentially undetectable (19) to

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Abbreviations used in this paper: tg, transgenic; AFP, amyloid precursor protein; RA, retinoic acid; PKC, protein kinase C; CXC chemokine receptor; AD, Alzheimer’s disease; CA, cornu ammonis.

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very low levels of the C5aR (20–22). Expression of the receptor in these studies was assessed by in situ hybridization and by ability to bind radiolabeled C5a.

Functional consequences of C5a binding to neuronal C5a receptors have been explored with human and mouse neuroblastoma cell lines, which have been shown to express the C5aR in RT-PCR and Northern blot analyses (22, 23). These experiments have shown that C5a and an oligomerized C5a peptide trigger modest and transient increases in intracellular calcium levels in human neuroblastoma cells (23) and trigger increased nuclear c-fos expression and induce apoptotic death of human neuroblastoma cells (23). It has also been reported that C5a protects against glutamate-induced neuronal apoptosis in vitro and in vivo (24). The latter findings are in accord with the finding that C5a-deficient mice exhibit heightened hippocampal neurodegeneration in response to injection of kainic acid, another excitotoxin (25), and that coinfusion of C5a-deficient mice with C5a in combination with kainic acid markedly reduced kainic acid-triggered neurodegeneration in vivo (24).

Although it is evident from the studies cited above that neurons can be induced to express C5a receptors, it is not clear whether neurons in normal brains constitutively express C5a receptors. The present studies were conducted to evaluate the possible presence and function of C5a on neuronal subsets in situ in normal brains and on primary explanted neurons in culture. Additionally, C5aR expression and function during neuronal differentiation was evaluated using neuroblastoma cells as a model system. The results show that normal neurons possess functional C5aR receptors. They further reveal that C5a has novel and distinct actions on undifferentiated and differentiated neuroblastoma cells.

Materials and Methods

Human brain tissue

Normal human brain tissue from aged nondemented individuals (78.8 ± 8.3 years in age) with very short autolysis times (2.8 ± 0.9 h) was kindly supplied by Thomas Beach (Civin Laboratory for Pathology Studies, Sun Health Research Institute, Sun City, AZ). Frozen 1-cm-thick coronal sections from the superior frontal gyrus and hippocampus were obtained at autopsy and stored at −80°C. The frozen blocks were sectioned (5 µm) on a cryostat, thaw mounted, and stored at −80°C until use. Immediately before use, sections were fixed in 1:1 methanol/acetone for 1 min at −20°C.

Mouse brain tissue

Normal mice (C57BL/6), micetg for human amyloid precursor protein (APP) (26), and micetg for HIV gp 120 (27) were sacrificed and mice mammary tumor virus reverse transcriptase (Life Technologies). PCR was conducted for 30 cycles using 5'-CATCTCGTCTACACTATGAG-3' as the upstream primer and 5'-ATGCAGCTTGTAGTGGCAG-3' as the downstream primer. These primers, derived from a sequence in exon 2, amplify a 530-bp fragment of C5aR, as previously described (11). Products were visualized with ethidium bromide.

Western blotting studies

Cell lysates (5% SDS, 10 mM EDTA, and 100 mM Tris, pH 6.8) were boiled (20 min), centrifuged, and the supernatants saved at −80°C until use. Samples were subjected to SDS-PAGE analyses on 12.5% gels and electrotransferred to polyvinylidene difluoride membranes. After blocking (5% milk, 2 h), the membranes were incubated overnight at 4°C with anti-C5aR Abs with rocking. After washing, bands were visualized after incubation with alkaline phosphatase-conjugated secondary Ab for 2 h, followed by a chemiluminescent detection solution (Pierce, Rockford, IL). The membranes were then exposed to x-ray film.

Immunohistochemical studies

After fixation, brain sections were washed three times, incubated in 50% methanol with 0.5% H2O2 for 5 min to block endogenous peroxidase activity, and then blocked with 3% horse serum for 2 h. The sections were incubated with primary Ab for 24 h at 4°C and then with peroxidase-conjugated secondary Abs as per kit directions (ABC Elite kit; Vector Laboratories, Burlingame, CA). Development was with diaminobenzidine with metal enhancer (Sigma). The sections were counterstained with hematoxylin and/or eosin (VWR Scientific Products, Willard, OH).

Fluorescent microscopes

Recombinant human C5a, a C5a agonist peptide (Sigma), or BSA (Sigma) were conjugated to carboxylate-modified 1 µM fluorescent microscopes (Molecular Probes, Eugene, OR) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Unbound amines were blocking with 1% BSA.

Tissue staining with fluorescent microscopes

Washed C5a, C5a agonist peptide, or BSA-conjugated beads were incubated with aceton/methanol fixed, 3% horse serum blocked sections for 24 h with constant shaking at 4°C. After thorough washing, the sections were viewed under a fluorescent microscope. In some experiments, the sections were incubated with a five times increased amount of recombinant C5a before incubation with the C5a-conjugated beads.

Protein kinase C (PKC) assays

Cytosolic extracts of neuroblastoma cells were incubated with recombinant C5a for varying periods of time before addition of an anti-PKC Ab (Upstate Biotechnology, Lake Placid, NY). The immunoprecipitates were washed and incubated with a specific PKC substrate using the PepTag assay (Promega, Madison, WI). Phosphorylated peptides were separated by agarose gel electrophoresis.

Gel shift assays

Nuclear fractions from C5a-stimulated neuroblastoma cells were incubated with a canonical NF-κB consensus binding probe and subjected to gel shift analyses as previously described (29).

Aβ toxicity assays

RA-differentiated SH-SY5Y neuroblastoma cells were incubated at 1 × 105 cells/ml in serum-free medium in the presence and absence of 100 ng/ml recombinant human C5a (Sigma). Twenty-four hours later, 5 µM...
Aβ(1–42) (California Peptide Research, Napa, CA) was added to the cultures. The Aβ peptide was aggregated as previously described (29). Mitochondrial activity (i.e., cell viability) was measured 48 h later using the MTT assay (Sigma).

**Ca²⁺ imaging studies**

Fourteen-day primary rat hippocampal cultures prepared from embryonic rat pups as previously described (30) were loaded with fura 2 (Molecular Probes). The coverslip cultures were incubated with 100 nM recombinant human C5a (Sigma), and calcium fluxes in individual neurons were digitally imaged and analyzed as previously described (30). In some experiments, the cells were incubated with a specific C5a antagonist peptide (Bachem) for 2 min or with pertussis toxin (Sigma) at concentrations of 900 nM and 500 ng/ml, respectively, before the addition of recombinant human C5a.

**Results**

**Constitutive expression of the C5aR on human neuroblastoma cells**

Undifferentiated and dividing as well as terminally differentiated SH-SY5Y and LAN-5 human neuroblastoma cells expressed the...
C5aR, since C5aR exon 2-specific primers (11) generated a 539-bp band in RT-PCR studies (Fig. 1A). Western blotting studies with undifferentiated and differentiated SH-SY5Y cells, as well as polymorphonuclear leukocytes included as a positive control, yielded a single band with an apparent Mr of 50,000, consistent with published reports for the neutrophil and astrocyte C5aR (11, 31) (Fig. 1B). As is apparent, there was no obvious difference in C5aR expression between undifferentiated and differentiated neuroblastoma cells at either the mRNA or protein levels.

Confocal microscopy was also used to evaluate the expression and distribution of the C5aR on undifferentiated and differentiated neuroblastoma cells. The receptor exhibited a patchy distribution on the membrane of undifferentiated SH-SY5Y neuroblastoma cells (green, Fig. 1C). There is little overlap in distribution with tubulin, which is red in the figure. With differentiation, the C5aR was found to be expressed on dendrites and to exhibit increased expression in synaptic regions (Fig. 1D, inset). Comparable results were obtained with LAN-5 cells (data not shown).

**Constitutive expression of the C5aR on subsets of human and murine neurons in the CNS**

The cell bodies of subsets of neurons in brains from aged non-demented individuals as well as Alzheimer’s disease (AD) patients were strongly reactive with Ab against the human C5aR. These include pyramidal neurons of the cornu ammonis (CA) subfields of the hippocampus (Fig. 2A), granular neurons of the dentate gyrus (data not shown), some pyramidal cells in the cortex (Fig. 2, B and C), and Purkinje cells in the cerebellum (Fig. 2D). Neuronal processes were also stained with the Ab (Fig. 2, B and C). Comparable reactivity was observed in aged normal (Fig. 2B) and AD (Fig. 2C) brains. No staining was observed when preimmune serum was used in place of the primary Ab (Fig. 2E).

Qualitatively identical results were obtained for the C5aR in the brains of normal mice using Ab to the murine C5aR. These included pyramidal neurons in the CA subfields of the hippocampus (Fig. 3A), granular neurons in the dentate gyrus (data not shown), pyramidal neurons in the neocortex (Fig. 3C), and Purkinje cells in the cerebellum (Fig. 3D). Not all neurons were positive for the C5aR, even in areas that were predominantly C5aR positive, as seen for C5aR-bearing neurons in the CA subfield in the posterior hippocampus which were restricted to the inner portion of the layer of neurons (Figs. 3, A and E). No staining was observed when the primary Ab was omitted (Fig. 3B). Comparable C5aR neuronal reactivity was observed in the brains of mice expressing a mutant human APP as a transgene in neurons, which represents a model for AD (26) (data not shown).

Since considerable variability was observed in these immunohistochemical studies with frozen tissue sections, in situ hybridization studies were conducted with labeled murine C5aR-specific riboprobes to confirm expression of the receptor mRNA in the murine brain. Neurons in the dentate gyrus (Fig. 4A), cortex (Fig. 4B), and cerebellar Purkinje neurons (Fig. 4C) reacted with the

**FIGURE 3.** Immunohistochemical analyses of the expression of the murine C5aR on neurons in the brains of normal mice. A, Expression of the murine C5aR on pyramidal neurons in the CA subfields of the hippocampus. B, Control with primary Ab omitted. C, Expression of the C5aR on pyramidal neurons in the neocortex. D, Expression of the C5aR on Purkinje cells in the cerebellum. E, Differential expression of the C5aR on pyramidal neurons in the CA hippocampal subfields of the posterior hippocampus. Magnification: A, B, and D, ×100; C and E, ×200.

**FIGURE 4.** In situ hybridization analyses of the expression of murine C5aR mRNA on neurons in the brains of normal mice using C5aR-specific antisense and sense probes. A and B, C5aR expression on murine neurons in the dentate gyrus. A and D, C5aR expression on pyramidal neurons in the neocortex. E and F, C5aR expression on cerebellar Purkinje neurons. A, C, and E were reacted with antisense probes, and B, D, and F with the corresponding sense probes. Magnification: C and F, ×10; A and D, ×100; B and E, ×200.
levels in hippocampal neurons, as shown in Fig. 6. 

The neuronal C5aR binds C5a: studies with fluorescent C5a microspheres

The ability of neuronal C5a receptors in the murine brain to bind C5a was evaluated next. In these experiments, postfixed frozen tissue sections were incubated with fluorescent microspheres bearing covalently bound recombinant C5a. Neurons in the murine dentate gyrus and CA1–CA3 subfields of the hippocampus of normal (Fig. 5, A and D), aged mutant human APP tg (Fig. 5B), and HIV gp120 tg mice (Fig. 5, E–G), many cortical neurons in a normal mouse brain (Fig. 5C), and cerebellar Purkinje neurons (data not shown) strongly reacted with the C5a-coated fluorescent microspheres. This is the same pattern as observed in the C5aR Ab and the C5aR riboprobe in situ hybridization studies described above. At higher power, strong reactivity of neurons in the dentate gyrus and CA1 subfield of the hippocampus was evident, with only occasional reactivity of interneurons in the stratum radiatum (Fig. 5D). Such higher power views showed specific binding of the beads to layers of neurons in the murine neocortex (Fig. 5E) and interesting patterns with increased bead binding to synaptic regions (Fig. 5, F and G). Young (10-wk) mice exhibited comparable reactivity, as shown in Fig. 5C. Specificity in these studies was assessed in three ways. First, tissue sections were preincubated with recombinant C5a, a treatment which greatly reduced binding of the microspheres to the tissue section, as seen in Fig. 5H, which represents a section adjacent to that shown in Fig. 5A, and Fig. 5I, which represents a section adjacent to that depicted in Fig. 5D. Second, fluorescent microspheres coated with albumin rather than C5a reacted poorly with the brain sections, as illustrated in Fig. 5J, which also represents a section adjacent to that shown in Fig. 5D. Third, beads coated with a C5a peptide reflecting the carboxy-terminal amino acids of the C5a peptide, which represents a C5a agonist (32), gave the same pattern as beads coated with intact C5a (Fig. 5K). Human brains also exhibited comparable reactivity with C5a-coated fluorescent microspheres (Fig. 5L). These results obtained in these control experiments indicate that the binding of C5a-coated fluorescent microspheres is specific.

The neuronal C5aR transduces intracellular signals

Ligand binding to the C5aR on neutrophils, monocytes, microglia, and astrocytes triggers intracellular signaling pathways leading to increased intracellular calcium levels (1, 2, 11, 33), activation of PKC, as well as to activation and nuclear translocation of the NF-κB transcription factor in granulocytes and monocytes (1, 2, 34). These three parameters were assessed in neuronal cells after addition of recombinant C5a. First, C5a-induced changes in intracellular calcium levels were assessed in individual rat hippocampal neurons in primary culture as a function of time. Recombinant human C5a triggered a biphasic increase in intracellular calcium levels in hippocampal neurons, as shown in Fig. 6B, whereas buffer alone elicited no reaction (Fig. 6A); each line in this figure represents the response of a single neuron. The first peak of increased levels of intracellular calcium levels occurred within a few seconds of C5a addition, while the second, slower increase peaked 60–75 s after C5a addition. The responses in individual neurons ranged between 80 and 350 nM calcium. Preincubation of the cells with a specific C5a antagonist peptide abrogated the responses to subsequently added C5a (Fig. 6C), and neurons preincubated with pertussis toxin also did not respond to C5a with increased intracellular calcium levels (Fig. 6C). Thus, primary neurons specifically respond to C5a with a biphasic increase in calcium levels triggered via a pertussis-sensitive Gi protein.

FIGURE 5. Binding of fluorescent microspheres bearing covalently bound C5a to the C5aR on neurons in murine brains. A. Binding of C5a microspheres to neuronal subsets in the murine dentate gyrus and CA1–CA3 subfields of the hippocampus of a normal mouse. B. Binding to C5a microspheres to neuronal subsets in the hippocampus of a mutant human APP tg mouse. C. Binding of C5a microspheres to cortical neurons in a 10-wk-old normal mouse. D. Binding of C5a microspheres to neurons in the dentate gyrus and CA1 subfield of the hippocampus of a normal mouse. E–G. Binding of C5a microspheres to neurons in the neocortex of HIV gp120 tg mice. H and I. Reactivity of C5a microspheres with the hippocampus of a normal mouse that was preincubated with C5a; sections are adjacent to those shown in A and D, respectively. J. Reactivity of fluorescent microspheres coated with albumin with the hippocampus of a normal mouse; the section is adjacent to that shown in D. K. Reactivity of microspheres coated with a C5a peptide agonist with the cortex of a normal mouse. L. Reactivity of C5a microspheres with cortical neurons in the brain of a nondemented person. Magnification: A and H, ×40; B, ×100; C–F and H–L, ×200; G, ×400.

Activation of PKC in SH-SY5Y human neuroblastoma cells was observed 5 min after C5a addition, and peak responses occurred 10–15 min after C5a addition (Fig. 7A). Activation and nuclear translocation of the NF-κB transcription factor also occurred after
addition of recombinant C5a to the neuroblastoma cells, as assessed by gel shift assays using a labeled NF-κB consensus probe (Fig. 7B). NF-κB activation was evident 5 min after addition of C5a to the cells, with the peak response occurring 10 min after addition of the ligand (Fig. 7B). The response was completely inhibited by a 100-fold excess of unlabeled probe. Of interest, C5a did not trigger changes in intracellular calcium levels in the neuroblastoma cells, indicating that PKC and NF-κB activation do not proceed via a Gi-coupled pathway in these cells. These findings document the functional integrity of the neuronal C5aR with respect to Gi-linked and Gi-independent intracellular signaling pathways.

C5a is neurotropic for undifferentiated human neuroblastoma cells

To our surprise, increasing concentrations of recombinant human C5a led to a dose-dependent increase in mitochondrial activity in undifferentiated SH-SY5Y cells in culture, as assessed by the MTT assay. Ten nanomolar C5a and 100 nM increased mitochondrial activity of the cells by 55 and 92%, respectively, when assessed 7 days after addition of C5a (Fig. 8A). Since spontaneous death of the actively dividing undifferentiated cells was extremely low, the apparent increase in viability in the MTT assay was due to increased cell division. This mitogenic activity of C5a for undifferentiated dividing neuroblastoma cells was strikingly evident upon morphological examination, which revealed large dense clumps of dividing cells 24 h after addition of C5a to adherent dividing SH-SY5Y cells (Fig. 8B). Comparable results were obtained with short-term (24 h) RA differentiated cultures (Fig. 8C).

In contrast, MTT assays showed that C5a produced no change in the viability of RA-differentiated SY-SH5Y cells (Fig. 8A). Consistent with these findings, no discernible morphological changes in RA-differentiated SH-SY5Y cells occurred in response to C5a (data not shown). Thus, C5a does not alter the viability of terminally differentiated cells.

C5a protects differentiated human neuroblastoma cells from Aβ-mediated neurotoxicity

C5a has been found to protect neurons from glutamate-mediated neurotoxicity both in vitro and in vivo (24, 25). In the present studies, the potential ability of C5a to protect neurons from the toxic actions of fibrillar Aβ was evaluated. Terminally differentiated SH-SY5Y cells were pretreated with varying concentrations of recombinant C5a for 24 h before the addition of 5 μM preaggregated Aβ1–42. Viability of the cultures was assessed by the MTT assay 48 h later. C5a increased the viability of the cells in a dose-dependent manner (Fig. 9). Clear-cut protection from the cytotoxic actions of Aβ was observed with 10 nM C5a, and 1 μM completely prevented Aβ-mediated neurotoxicity. These data indicate that the neuronal C5a receptor transduces intracellular signals which enhance the survival of differentiated human neuroblastoma cells.

Discussion

Previous studies have clearly shown that neurons in murine brains undergoing inflammatory responses in response to infection, cytokine expression or injection, trauma, or excitotoxin administration express the C5a receptor (19–22). However, these reports have not provided definitive information about the expression of the C5aR on neurons in normal brains or its distribution. These aspects, as well as functional studies of constitutively expressed neuronal C5a receptors, are addressed in the current studies.

In the present experiments, Abs generated to similar aminoterminal protein sequences of the human and murine C5a receptors...
reacted with neuronal subsets in the brain of normal adult humans and mice. These included granular neurons of the dentate gyrus, pyramidal neurons of the hippocampal CA1–CA3 subfields, many pyramidal cortical neurons, and cerebellar Purkinje cells. Although not shown here, another Ab generated to the same amino-terminal residues of the human C5a receptor failed to react with human neurons, although it did react with activated glial cells in the brain. The reactivity of this Ab and studies with it are being reported separately. Because of this variability in Ab reactivity, we used C5a-coated microfluorospheres as an Ab-independent method to evaluate neuronal C5aR expression in normal brains. Additionally, we conducted in situ hybridization experiments to detect C5aR mRNA. All three approaches yielded the same pattern of reactivity with various subsets of neurons. From these studies, therefore, it is clear that certain neuronal subsets in normal human and murine brains constitutively express easily detectable levels of the C5aR. Although the Ab-based experiments suggest that the C5aR is expressed intracellularly in neurons, the experiments with the C5a-coated fluorescent microspheres indicate that the receptor is also expressed on the surface of the neurons; furthermore, these experiments demonstrate that the receptor binds C5a. Experiments with explanted rodent hippocampal neurons in primary culture showed that the receptor is functionally competent and transduces intracellular signals, leading to a biphasic, rapid increase in intracellular calcium levels.

The biological significance of functional C5a receptors on subsets of normal neurons in the adult brain is far from clear. However, certain aspects of the biology of the complement system, when interpreted in the context of the constitutive expression of its components in the CNS, along with emerging experimental data, suggest possible roles for neuronal C5a receptors. For example, C5a, the only described ligand for the C5a receptor, is probably only generated during complement activation, since numerous physiological trypsin-like enzymes, such as thrombin, plasmin, and elastase, are unable to cleave the specific bond in C5 which generates C5a (35). This, coupled with the increased neuronal expression of the C5aR (19–22) and the various complement components of the activation and membrane attack pathways during CNS inflammatory conditions (in response to neurotoxin injection, in experimental meningitis, and in Alzheimer’s disease) (36–40), strongly suggests that the functions of the neuronal C5aR in inflamed brains are concerned with neuronal responses to complement activation.

It is reasonable to assume that the constitutively expressed neuronal C5aR in normal adult brains is also concerned with neuronal responses to the activated complement system. The surprising constitutive expression of essentially all of the components of the classical and membrane attack pathways of the complement system in normal brains (41) makes it likely that complement activation can occur in normal brains. The constitutive synthesis of most and probably all of the activation components of the system is of considerable interest, since it indicates that the normal brain is “primed” to respond with complement activation to appropriate stimuli. Clearly, the identity of complement-activating stimuli in the normal brain is not known, except for the rare entry of infectious agents. Potential complement activators could, however, include products of degrading cells or of cellular remodeling processes, cellular constituents released as a result of microscopic tears in blood vessels occurring during trauma, products of oxidative processes, or cellular metabolites. It is undoubtedly relevant that neuronal subsets, notably dentate gyrus granule cells, hippocampal pyramidal hilar cells, and cerebellar Purkinje cells also constitutively express receptors for the C3a complement activation peptide and a number of chemokine receptors, including CXC chemokine receptor (CXCR) 2, CXCR3, CXCR4, CCR1, CCR3, CCR4, CCR5, CCR9, and CX3CR1 (42–47). The existence of primed proinflammatory systems in the brain probably reflects the essential role of the innate immune system in protecting the brain from damage produced by foreign agents, trauma, altered cells, abnormal tissue products, and degrading cells.

With regard to the biological function of neuronal C5a receptors in responding to complement activation in the normal brain, it is possible, although in our view unlikely, that C5a binding to neuronal C5a receptors leads to neuronal activation, migration, and mediator release. It is thus probable that neuronal C5a receptors subserve different functions than the C5a receptors on myeloid cells. Numerous novel actions for neuronal C5a receptors have
been postulated, including roles in dendrite outgrowth, cytoskeletal reorganization, synthesis of neurotrophins, and clearance of C5a (48). Another possibility, supported by the present experiments in which C5a protected differentiated human neuroblastoma cells from neurotoxic damage mediated by the amyloid Aβ fragment, is that the neuronal C5αR is neuroprotective. Additional supportive evidence in favor of this hypothesis includes the findings that C5-deficient mice, which cannot generate C5a, exhibited heightened hippocampal degeneration in response to intraventricularly infused kainic acid, as compared with complement sufficient mice (25, 49), and that confusion of C5a markedly reduced neurodegeneration (22). Additionally, C5a protected primary neurons in C5-sufficient mice from glutamate toxicity (22). Further supporting a neuroprotective role are our findings that recombinant C5a rapidly activated PKC and the NF-κB transcription factor in human neuroblastoma cells. Signaling pathways mediating activation of both factors have been found to be involved in protection of neurons from toxic damage to diverse agents, as well as cell death in multiple systems (50–55).

Because of the above findings and our data, we, like others (24, 25, 56), postulate that the binding of C5a to neuronal C5a receptors protects the neurons from injury produced by excitotoxins, reactive oxygen products, and other toxic substances generated during local inflammatory processes. Local inflammatory processes may be common in the brain, in view of the presence of the constitutively expressed components of the complement system, which require only activation to trigger a self-amplifying sequence of events mediated by the actions of complement activation products on various cell types in the vicinity of the activation process. In the brain, as elsewhere in the body, such complement fragment dependent reactions would stimulate the synthesis and release of proinflammatory cytokines, secondary mediators, cellular constituents, and toxic products which in turn would act on cells in the immediate vicinity to stimulate additional cellular responses and thereby augment local inflammatory processes. It seems imperative that neurons would possess protective mechanisms to prevent injury mediated by toxic agents produced during local inflammatory processes occurring in proximity to their cellular processes. A critical role for C5a in such neuroprotection is reasonable, since C5a is readily generated during complement activation processes which lead to inflammation. Of interest, several other cytokines exhibit neuroprotective actions, including IL-6 and TNF (53, 57).

A neuroprotective role for neuronal C5a receptors clearly does not preclude other functions for such receptors. Ligation of C5a receptors on myeloid cells, astrocytes, and microglia stimulates signaling pathways which alter cytoskeletal structure and stimulate the motile machinery of the cells. In neurons, comparable actions could be envisaged as facilitating axon guidance and synapse formation. Such actions, which have not been evaluated, could be particularly important in CNS development. Perhaps related, RANTES, a chemokine which binds to CCR1, CCR3, and CCR5, was found to stimulate embryonic mouse dorsal root ganglia neurons to migrate directionally and to develop markers associated with sensory neurons (46). Also, mice genetically engineered to lack stromal cell-derived factor-1α, or its receptor CXCR4, exhibited abnormal migration of developing neurons leading to altered cerebellar architecture (58, 59).

In our studies, recombinant C5a triggered augmented cell division of undifferentiated human neuroblastoma cells in culture, a very different effect than the neuroprotective actions observed with the terminally differentiated neuroblastoma cells. Differential activity with undifferentiated vs differentiated cells is perhaps not surprising, since different actions would undoubtedly be required during nervous system development, as compared with maintaining efficient function of the fully differentiated nervous system. This neurotropic activity of C5a for undifferentiated neuroblastoma cells represents a previously undescribed action for this chemokine-like complement activation fragment. Perhaps reflecting similar roles for chemokines, IL-8, which binds to CXCR1 and CXCR2, augmented cell division of embryonic rat hippocampal neurons in mixed cultures (60).

Finally, the neuronal C5αR could regulate important signal transduction pathways in neurons. In this regard, evidence for C5α regulation of glutamate receptor functions in neurons has been presented (25, 49, 56, 61). As with other suggested C5α functions, it has been reported that two other chemotactic peptides, IL-8 and growth-related oncogene-α2, which bind to CXCR1 or CXCR2 and CXCR2, respectively, alter signal transduction in Purkinje neurons (62). Reflecting possibly similar mechanisms, murine stromal cell-derived factor-1α, RANTES, macrophage-derived chemokine, and fractalkine, chemokines which bind to CXCR4, CCR4, and CX3CR1, regulate synaptic transmission of embryonic rat hippocampal neurons (46).

These data document the constitutive expression of the C5αR on neurons in normal human and murine brains. Studies presented in recent publications indicate that neurons also possess specific receptors for multiple chemokines. Glial cells and neurons also possess the ability to synthesize the ligands for these receptors. Emerging functional studies indicate that these neuronal chemotactic peptide receptors subserve very different actions than the proinflammatory actions observed with other cell types. Additionally, the functional consequences of engagement of neuronal C5α receptors differs with the stage of neuronal development. These findings have implications for the understanding of neuronal C5α receptors in neuronal development, in neuronal homeostasis, and also in neuroinflammatory conditions such as Alzheimer’s disease.

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