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C3aR inhibition reduces neurodegeneration in experimental lupus

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Abstract

Complement activation is an important aspect of systemic lupus erythematosus. In this study we investigated the role of C3a/C3a receptor (R) signaling in brains of the lupus model, MRL/*lpr* mice, by treating the mice with C3aR antagonist (a) from 13 to 19 weeks of age. C3aR mRNA (0.2 ± 0.027 versus 0.56 ± 0.19) and protein (0.16 ± 0.09 versus 0.63 ± 0.19) expression was increased in MRL/*lpr* brains compared with MRL+/+ controls. Apoptosis, a key feature in lupus brain, was significantly reduced by C3aRa treatment, as assessed by DNA laddering, TUNEL staining and caspase3 activity (48% of MRL/*lpr* mice). mRNA expression of proinflammatory molecules that cause apoptosis, TNF α (0.33 ± 0.07 versus 0.15 ± 0.1), MIP2 (3.8 ± 1.3 versus 1.7 ± 0.6), and INF γ (4.8 ± 1.0 versus 2.07 ± 1.28) are reduced in MRL/*lpr* brains with C3aRa treatment. In line with these results, Western blotting demonstrates the significant increase in phosphorylation of survival molecules Akt and Erk, decrease in PTEN and reduced iNOS expression. INF γ receptor (R) and AMPA-GluR1 co-localized, and concomitant with reduced INF γ R expression, AMPA-GluR1 expression was also decreased by C3aR antagonist. All of these variables that modulate neuronal excitability and regulate synaptic plasticity are C3aR dependent in the MRL/*lpr* brains and suggest a potential therapeutic role for C3aR inhibition in CNS lupus.

Keywords

anaphylatoxins; brain; complement; inflammation; systemic lupus erythematosus

Introduction

Central nervous system (CNS) involvement occurs in 14–75% of systemic lupus erythematosus (SLE) patients and is associated with increased mortality.¹ The exact underlying mechanism causing the CNS pathology remains unknown. The complement system is an innate immune mechanism that goes awry and contributes to increased or sustained inflammation and damage in SLE.^{2–6} Once activated, the complement system generates the anaphylatoxins, C3a and C5a.^{7–9} The systemic levels of anaphylatoxins correlate with CNS disease in SLE patients.¹⁰ Our previous studies demonstrate that complement inhibition by the upstream complement regulator, Crry, which inhibits the convertases, or by the absence of factor B, a ‘key’ protein of the alternative pathway significantly alleviates CNS disease in the experimental model, MRL/*lpr* mice.^{11,12} Given that inhibition of C3 convertases prevents generation of anaphylatoxins and other downstream products, it is conceivable that the therapeutic effects we observed were secondary to its effect to limit generation of C3a. Complement proteins can be neuroprotective or neuroinflammatory depending on the setting. C3a prevented neither

serum deprivation-induced apoptotic neuronal death, nor AMPA/kainate-mediated excitotoxicity. However, in mixed cultures of neurons and astrocytes, C3a dose-dependently protected neurons against NMDA toxicity.¹³ Hence, in this study we assessed the role of C3a in CNS lupus using a small molecule inhibitor and the MRL/MpJ-*Tnfrsf6^{lpr}* (MRL/*lpr*) strain, mice that are felt to closely reflect that which occurs in human SLE, including the neuropsychiatric manifestations.¹⁴

C3a binds to its G-coupled receptor, C3aR, located on different cell types¹⁵⁻¹⁷ and induces a wide range of inflammatory and immune effects.^{8,18,19} The anaphylatoxins can aggravate or exacerbate inflammation and apoptosis, key events in experimental lupus brain.²⁰ When increased in circulation, it can lead to increased infiltration of proinflammatory cells, activation and proliferation of glial cells,²¹ generation of cytokines and chemokines and thereby apoptosis of nearby cells such as neurons. Apoptosis observed in MRL/*lpr* mice^{11,22} can be regulated by the PI3K/Akt pathway^{23,24} and its negative modulator, PTEN (phosphatase and tensin homolog deleted on chromosome ten)^{25,26} by acting on diverse downstream targets, including Bad and NF- κ B.^{27,28} Downstream targets of NF- κ B such as IL-1 β , RANTES, MCP-1 and MIP-2 can mediate CNS functioning²⁹ and play a role in neuropsychiatric (NP)-SLE.³⁰ Caspase-3, the cysteine protease that executes apoptosis, was recently shown to directly cleave AMPA-GluR1 and modulate neuronal excitability, regulate synaptic plasticity and neuronal survival. AMPA-GluR1 forms unique calcium permeable complexes with INF γ R on astrocytes. The presence of INF γ on one of the lupus susceptibility loci, its elevated mRNA expression and its ability to cause behavioral disturbances in both humans and animals suggest that the γ INF pathway is important for the disease pathogenesis. The goal of this study was to determine the role of C3a/C3aR signaling on these variables.

Our results demonstrate for the first time that C3a produced on complement activation plays an important role in inducing the pathology seen in experimental CNS lupus. C3a acting through its receptor, C3aR, caused apoptosis and gliosis and altered neurotransmission in experimental lupus brain. Although further studies are needed to understand the signaling pathways involved, our results identify C3aR as a possible promising therapeutic target for lupus and other neurodegenerative diseases.

Materials and methods

Reagent and antagonist

All chemicals were procured from Sigma (St Louis, MO, USA) unless otherwise stated. Antibodies used were anti-C3aR, rabbit anti-GFAP (Dako, Carpinteria, CA, USA), rabbit anti-AMPA GLuR1 (Santa Cruz, USA), goat anti-INF γ R (Pharmingen), and anti-PCNA. Rabbit anti-Akt, anti-PTEN, and anti-Erk (Cell Signaling) at 1:1000 dilution were used for Western blotting. FITC labeled donkey anti-rabbit (1:300; Jackson ImmunoResearch, USA), FITC anti-goat, and FITC anti-chicken (1:250; Sigma) were used as secondary antibodies.

C3aRa (N2-[(2,2-diphenylethoxy)acetyl]L-arginine) was obtained from Calbiochem and has an IC₅₀ = 200 nM for the mouse C3aR. C3aRa has a short half-life (1.5 h) and therefore was administered continuously using subcutaneously placed osmotic pumps (Alzet model 2001; Durect). The dose of 60 mg/kg/day C3aRa was shown to be effective based on increased survival in these mice.³¹ In the same studies, C3aR blockade did not alter the autoimmune features in MRL/*lpr* mice such as circulating anti-dsDNA antibodies and immune complexes.

Treatment

To determine the role of C3aR in CNS lupus, MRL/*lpr* mice obtained from Jackson Laboratories were treated with the selective non-peptide antagonist of C3aR, C3aRa dissolved

in 50% DMSO from the time of clinical onset of disease at 13 weeks of age to the time of LD₅₀ at 19 weeks, when they were killed.³¹ Control mice were treated with non-specific garbled peptide in 50% DMSO from 13–19 weeks of age. The number of mice used in each group was large due to the pathological variation that may occur between them. Twenty-seven male MRL/*lpr* mice (The Jackson Laboratory) were randomly divided into two groups to receive C3aRa ($n = 13$) or vehicle alone ($n = 14$). Osmotic pumps were replaced weekly in order to maintain the circulating concentration of C3aRa constant. These studies were approved by the University of Chicago Animal Care and Use Committee.

Immunofluorescence microscopy

Cryosections (7 μ m) obtained from these mice were fixed in 4% formaldehyde followed by 1:1 ether–ethanol and 95% ethanol. The sections were then exposed to antibodies to visualize the proteins of interest. Antibodies were used at a dilution of 1:100. Briefly, sections were incubated overnight in rabbit anti-C3aR, rabbit anti-MAP2, rabbit anti-AMPA GluR1, goat anti-INF γ R, chicken anti-vimentin, or rabbit anti-GFAP. This was followed by incubation (2 h) in FITC donkey anti-rabbit (1:300) second antibody. Alexa-547 coupled PCNA and DAPI for nuclei were used for direct labeling. All assays included negative controls where the primary antibody was omitted. Upon completion of staining, all slides were coded so that the examiner was blind to the treatment group.

Ligase-mediated polymerase chain reaction

Brains were harvested and DNA laddering was amplified and detected by LM-PCR as described previously.^{20,32} DNA isolated from each animal was ligated with the supplied primer targets for 18 h at 16°C. The ligated DNA was then used as the substrate for PCR, using supplied primers and Advantage DNA polymerase (Clontech Laboratories, Palo Alto, CA) for 23 cycles of 94°C for 1 min and at 72°C for 3 min. The reaction product was electrophoresed through a 1.2% agarose gel, and ethidium bromide-stained bands were detected with UV light illumination.

Assay of caspase-3 activity

Brains were homogenized in cell lysis buffer (25 mM HEPES pH 7.4 buffer containing 2 mM DTT, 5 mM EDTA, and 10 mM digitonin).²⁶ Lysates were incubated on ice for 15 min and protein concentrations were determined in the supernatant using the BCA assay. The supernatants were stored at –80°C. Proteins (50 μ g) were incubated at 37°C with assay buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM dithiothreitol, 1 mM EDTA, 10% glycerol) and 200 mM Ac-DEVD-pNA (Biomol, Plymouth Meeting, PA). Hydrolysis of the DEVD-AFC substrate was followed for 15 min by fluorometry of the released AFC (excitation 400 nm, emission 505 nm) and activity was calculated from the slope. The addition of the caspase-3 inhibitor Ac-DEVD-CHO (0.1 mM; Biomol) to the reaction mixture was used to confirm the specificity of the assay.

Quantitative reverse-transcriptase polymerase chain reaction

RNA was isolated from brains using TRIzol reagent (Life Technologies, Grand Island, NY) and qRT-PCR performed as described previously.³³ Real-time qRT-PCR for TNF- α , CXCL2/MIP-2, IFN- γ , and ICAM-1 were performed on RNA isolated from brain. All traces of genomic DNA were eliminated with RQ1 DNase (Promega, Madison, WI) at 37°C for 30 min. cDNA was generated from RNA using random hexamers with the SuperScript first-strand synthesis kit (Life Technologies), and qPCR was performed using a Smart Cycler (Cepheid, Sunnyvale, CA) and the SybrGreen intercalating dye method with Hot-Star DNA polymerase (PE Applied Biosystems) according to the manufacturer's instructions. Each reaction (25 μ l) was conducted with 1 μ l TaqMan Master Mix (PE Applied Biosystems, Foster City, CA), 3 μ l sample or

standard cDNA and primers at 200 nM each. PCR was conducted with a hot start at 95°C (5 min), followed by 45 cycles of 95°C for 15s and 60°C for 30s. For each sample, the number of cycles required to generate a given threshold signal (C_t) was recorded. Using a standard curve generated from serial dilutions of splenic cDNA, the ratio of gene expression relative to GAPDH expression was calculated for each experimental and control animal. Primers were synthesized by Integrated DNA Technologies (Coralville, IA) and probes by Synthegen (Houston, TX). The sequences of primers/probes are given in Table 1.

Western blotting

Tissue samples were homogenized in cold RIPA buffer (with a protease inhibitor cocktail from Sigma). Protein content was measured using the BCA reagent and bovine serum albumin as standard. We electrophoresed 30 µg of protein by SDS-PAGE and electroblotted to PVDF membranes for detection. Membranes were blocked with Tris-buffered saline (TBS) containing 5% dry milk, rinsed with TBS plus Tween (T, 0.05%), then incubated with primary antibodies: anti-Akt, anti-PTEN, and anti-Erk in TBST and bovine serum albumin (0.2%) overnight at 4°C. The primary antibody was removed, membranes washed and peroxidase-labeled anti-rabbit secondary antibody added for 2 h. Following further washes with TBST, bands were visualized with an ECL Western Blotting Analysis System (Amersham, Pierce, Rockford, IL, USA). The Western blots were quantitated using Image J software.

Statistical analyses

Data are expressed as mean $\pm$ SEM and were analyzed using Minitab (version 12; Minitab) software. For the comparison between two groups, *t* testing was used for parametric data, and Mann-Whitney testing was used for non-parametric data. Significance was determined as $p < 0.05$.

Results

C3aR expression is up-regulated in brains of MRL/lpr mice

C3aR mRNA expression, assessed by real-time PCR, was markedly up-regulated in brains of MRL/lpr mice relative to the age-matched, 19-week-old, MRL/+ controls ($p < 0.05$). The observed increase in C3aR mRNA was translated into protein as shown by Western blotting ($p < 0.05$, Figure 1A). Representative cortical sections were stained for C3aR. C3aR was observed to be membrane-bound and localized on the surface of the cells (Figure 1B, A). Higher expression of C3aR was present in MRL/lpr mice (Figure 1B, B) compared with controls Figure 1B, A. C3aR is present on neurons in brains of MRL/lpr mice as indicated by co-staining with anti-MAP2 antibody (Figure 1C).

C3aR inhibition reduces neuronal cell death in MRL/lpr mice

Our earlier studies demonstrate complement-dependent neuronal cell death in brains of MRL/lpr mice compared with their MRL^{+/+} counterparts.^{11,20} DNA fragmentation (LM-PCR) techniques show reduced laddering in brains of MRL/lpr mice when treated with C3aRa (Figure 2A, a). Dual staining with specific cell-marker NeuN demonstrates that the main TUNEL-positive cells were neurons (Figure 2b, c and d). The TUNEL positive cells were observed in all tissue sections assessed and the figures shown are representative for the mice in each group. Caspase-3, a critical mediator of apoptosis, was increased in MRL/lpr mouse brains, as assessed by hydrolysis of the DEVD-AFC substrate. Treatment with C3aRa significantly reduced (48% of MRL/lpr mice, $p < 0.05$) caspase-3 activity in lupus brain (Figure 2B). These findings further support the concept of complement-dependent neuronal apoptosis and demonstrate the important role of C3a/C3aR signaling in causing neurodegeneration in lupus.

C3aRa treatment alters survival signaling pathways

Since Akt and MAPK/Erk pathways are essential for growth and survival of neurons, we evaluated the role of C3a in these pathways. Western blot analysis revealed that phospho (p)-Akt (phosphorylated at serine 473) was increased in mice treated with C3aRa ($p < 0.03$) compared with the untreated MRL/lpr mice. In contrast, PTEN, the negative modulator of p-Akt, was substantially reduced in C3aRa treated MRL/lpr mice ($p < 0.02$) (Figure 3). C3aRa treated mice had increased phosphorylation of Erk ($p < 0.02$) similar to that observed for Akt indicating that C3aR related (but Fas-independent) signals lead to apoptosis, which may involve PKB/Akt and Erk signaling pathways.

Inhibition of C3aR reduces the expression of inflammatory mediators in lupus brain

Increased infiltrating cells in MRL/lpr brains can induce an inflammatory response. In line with this, earlier studies showed reduction of neutrophil infiltration into brains of MRL/lpr mice reduced the severity of disease. However, our results show that C3aR inhibition caused a reduction in neutrophil number which did not reach statistical significance (data not shown). C3a binds to its G-coupled receptor, present on glial and infiltrating cells and initiates inflammatory functions. Since one of the predominant effects is the induction of cytokine signaling, we studied the mRNA expression of inflammatory mediators in the brains of these mice. IFN γ -, TNF α -, ICAM-1, and CXCL2/MIP-2 were measured to provide insight into the potential mechanism(s) that result from complement activation and thereby increased C3a expression. As shown in Figure 4, the expression of TNF α - (0.33 ± 0.07 versus 0.15 ± 0.1 , $p < 0.05$), MIP-2 (3.8 ± 1.3 versus 1.7 ± 0.6 , $p < 0.02$), and IFN γ - (4.83 ± 1.0 versus 2.07 ± 1.28 , $p < 0.02$) in control and C3aRa-treated groups, respectively, were significantly changed, while ICAM-1 (5.0 ± 1.6 versus 5.0 ± 2.3) remained unaltered in C3aR-inhibited lupus brain, indicating that the expression of these cytokines appears to be mediated, at least in part, through signals delivered through C3aR.

C3aRa treatment alters AMPA-GluR1 and INF γ signaling

Our earlier studies showed that AMPA-GluR1 is altered in CNS lupus while the studies of several others demonstrated that the INF-pathway is important for the disease pathogenesis. Immunofluorescence shows that expression of both the AMPA-GluR1 (red, Figure 5A and B) and INF γ R (green, Figure 5C and D) co-localize (merge, Figure 5E and F) and they are significantly reduced in brains of MRL/lpr mice treated with C3aRa (Figure 5B, D and F) compared with their untreated counterparts (Figure 5A, C and E).

C3aRa treatment reduces iNOS mRNA expression in lupus mice

IFN- γ is a potent inducer of NO, and can induce iNOS expression. In this study using qRT-PCR we examined and found that inhibition of C3aR significantly reduced iNOS mRNA expression in brains of lupus mice (Figure 6, $p < 0.05$).

C3aR inhibition reduces gliosis in MRL/lpr mice

Since INF γ R and AMPA-GluR1 were observed on astrocytes, we studied the involvement of these cells in lupus by immunostaining for glial fibrillary acidic protein (GFAP) and vimentin in brain sections. The astrocytes in MRL/lpr mice show marked reduction of GFAP and vimentin immunoreactivity, on C3aR inhibition (Figure 7B and D). Both GFAP and vimentin co-localized on the astrocytes. However, vimentin containing astrocytes exhibited fewer long and straight processes and was predominant around the microvasculature (Figure 7C, arrow). This was accompanied by decreased proliferation as indicated by PCNA staining (red, Figure 8) with DAPI in blue.

Discussion

The present study extends our previous observations and provides new insights on the role of complement in lupus brain. Our earlier studies showed that complement inhibition with Crry or factor B deletion exerted a positive effect and reduced both the pathological changes in the brain of lupus mice and the resulting behavioral alterations.^{11,20} The present study, designed to identify effective downstream therapeutic targets, demonstrates the potential of C3a as a strong therapeutic candidate. C3aR expression is increased in the brains of MRL/*lpr* mice, suggesting a role for this receptor and its ligand in lupus brain.

Gliosis can be beneficial or harmful and its manipulation could provide an appropriate environment for neuronal regeneration. Significant activation of astrocytes characterized by hyperplasia, hypertrophy, and increased GFAP content and vimentin expression occurs in MRL/*lpr* brain.³³ Astrocyte activation or astrogliosis was observed mainly in the hippocampus and cortical regions of the brain. In line with gliosis our results show C3a-dependent increase in proinflammatory cytokines. The expression of ICAM-1 which regulates the transport of cells across membranes is increased in MRL/*lpr* brains and was not affected by C3aR inhibition. Concomitantly, there was no significant change in the infiltration of neutrophils into brain. The increase of cytokines γ -INF, TNF α , and MIP2 observed in lupus brain could aggravate inflammation and cause apoptosis. Neuronal apoptosis is a key event in experimental lupus brain.¹¹ In a complex, systemic disease such as lupus, several factors could cause neuronal death including cytokines, oxygen free radicals, and autoantibodies. C3aR inhibition maintained the survival signaling molecules, including phosphorylated Erk, Akt (serine 473), and its negative modulator PTEN at normal levels in lupus brains. Since Fas protein is absent in MRL/*lpr* mice, it is conceivable that C3a regulates apoptosis through Akt and Erk signaling pathways independent of Fas, in the setting of lupus.

IFN and IFN-inducible gene signatures with elevated levels of mRNA are present in both lupus patients and mouse models, suggesting that they may play a role in the disease pathogenesis.^{34,35} IFN- γ Rs are expressed by both neurons and glia^{36,37} and form a unique, neuron-specific, calcium-permeable receptor complex with AMPA receptor subunit GluR1.³⁸ In addition, IFN- γ is a potent inducer of NO,³⁹ and can induce both calcium-dependent NO synthase in neurons (nNOS) or the inducible NOS (iNOS). NO can amplify the release of glutamate from presynaptic sites⁴⁰ and inhibit glutamate uptake by astrocytes concomitant with the increased glutamate levels observed in these mice.⁴¹ NO produced along with the phosphorylated AMPA-GluR1 can alter Ca(2+) influx, decrease ATP production, and cause neuronal toxicity. Increase in the transcription factor c-Fos is regulated by glutamate and indicates neuronal activation, astrocytic alteration, and stress in the brain. Future studies are required to decipher the crosstalk between apoptosis and gliosis following insult in these mice.

In this study, we have shown for the first time that C3a plays an important role in CNS lupus, signaling through its receptor C3aR. Inhibition of C3aR alleviated two key events, neuronal apoptosis and gliosis, in this complex setting. In addition, our studies suggest that the neuronal toxicity could be through the INF γ R–AMPA–GluR1 complex. Since this study has addressed the changes occurring globally in brain, further studies are required to understand the cellular localization of the signaling crosstalk in the lupus brain.

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Abbreviations

Akt/PKB	protein kinase B
C3aRa	(N2-[(2, 2-diphenylethoxy)acetyl]L-arginine)
CNS	central nervous system
GFAP	glial fibrillary acidic protein
LM-PCR	ligase-mediated polymerase chain reaction
MRL/ <i>lpr</i>	MRL/MpJ- <i>Tnfrsf6^{lpr}</i>
NP-SLE	neuropsychiatric systemic lupus erythematosus
PTEN	phosphatase and tensin homolog deleted on chromosome ten
qRT-PCR	quantitative reverse-transcriptase polymerase chain reaction
SDS	sodium dodecyl sulfate
SLE	systemic lupus erythematosus

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Figure 1.

C3aR expression is up-regulated in brains of MRL/*lpr* mice. (A) A significant increase in C3aR mRNA ($p < 0.04$) and protein expression ($p < 0.03$) occurred in brains of MRL/*lpr* mice compared with the congenic MRL/+ controls. mRNA expression of C3aR as determined by RT-PCR was normalized to GAPDH (arrow) and protein expression assessed by Western blotting was normalized to actin (arrow). (B) Representative brain sections stained with Alexa-488 labeled anti-C3aR indicate increased C3aR expression in brains of MRL/*lpr* mice (B) compared with MRL/+ controls (A). Inset is a cell at higher magnification (40 $\times$ under oil) showing C3aR receptor expression mainly on the cell-surface. (C) Representative brain sections co-stained with anti-C3aR (A, green) and anti-MAP2 (B, red) and merge (C) indicate C3aR expression in brains of MRL/*lpr* mice is localized on neurons. Inset are cells (arrow head) at higher magnification (40 $\times$ under oil).

Figure 2.

C3aR inhibition reduces apoptosis in experimental lupus brain. **(a)** Representative gel demonstrates increased apoptosis by LM-PCR in MRL/lpr brains (lanes 1–3). Inhibition of C3aR reduced apoptosis in lupus brains (lanes 4–6). Each lane represents an animal. Shown are representative samples from MRL/lpr mice treated with C3aRa and MRL/lpr mice treated with vehicle alone. Sections from brains of MRL/lpr mice were stained for neurons with anti-neurofilament Ab (c) and for TUNEL-stained apoptotic nuclei (b). Merge of images (b) and (c) show that the cells undergoing apoptosis are predominantly neurons (d). **(B)** Caspase-3 activity in brains of MRL/lpr mice is reduced by C3aR inhibition. The activity of caspase-3, as assessed by hydrolysis of DEVD-AFC substrate was significantly reduced to 50% in brains of MRL/lpr treated with C3aRa ($n = 13$) compared with control MRL/lpr mice ($n = 14$). Data are expressed as mean $\pm$ SD. * $p < 0.05$ compared with matched MRL/lpr mice.

Figure 3.

Anaphylatoxin-dependent Akt signaling pathway plays a key role in central nervous system (CNS) apoptosis in lupus. Phosphorylated Akt was increased ($p < 0.03$) and phospho-PTEN decreased ($p < 0.02$) in MRL/lpr mice treated from 13 to 19 weeks with C3aRa. Equivalent quantities of CNS protein from individual mice were subjected to SDS-PAGE and immunoblotted with specific Abs for p-Akt (serine 473), p-PTEN (serine 380), and phospho-Erk and normalized to actin. Blockade of the anaphylatoxin receptors led to increased p-Akt and -Erk and reduced p-PTEN. Shown are representative immunoblots from three separate experiments.

Figure 4.

C3a alters the expression of inflammatory mediators in MRL/*lpr* brains. mRNA expression of the inflammatory mediators TNF α , MIP2, γ -INF, and ICAM-1 in MRL/*lpr* mice treated from 13 to 19 weeks of age with C3aRa ($n = 13$) or vehicle ($n = 14$) were assessed by qRT-PCR. The expression of the mediators was significantly decreased in the brains of antagonist treated mice, $*p < 0.05$, compared with vehicle-treated control MRL/*lpr* mice except ICAM-1 expression which remained the same in both groups. Data presented as units are expression relative to 18S RNA measured in the same sample.

Figure 5.

Expression of INF γ R and AMPA-GluR1 are C3aR-dependent. Representative 7 μ m cortical cryosections stained for INF γ R (red) and AMPA-GluR1 (green) show that they co-localize in brain. Sections were counterstained with DAPI (blue). Both INF γ R and AMPA-GluR1 were highly expressed in MRL/lpr brain (A, C and E) compared with brains from C3aR-inhibited mice (B, D and F).

Figure 6.

Expression of iNOS is decreased in brains of MRL/*lpr* mice treated with C3aRa. As shown by qRT-PCR, iNOS mRNA expression was decreased in brains from MRL/*lpr* mice treated with C3aRa compared with MRL/*lpr* mice treated with vehicle alone. Data are shown as mean values from C3aRa ($n = 13$) or vehicle ($n = 14$) treated mice. * $p < 0.05$.

Figure 7.

C3aR antagonist treatment reduces astrogliosis, in the brains of lupus mice. Representative sections immunostained for GFAP and vimentin to determine the degree of astrocyte involvement. Sections from MRL/*lpr* mice treated with vehicle (A and C) or with C3aRa (B and D) are given here. Vehicle-administered mice had numerous GFAP (A) and vimentin (C)-labeled astrocytes compared with those treated with C3aRa (B and D). Photomicrographs were taken at 10X magnification with a Zeiss camera. All images are typical and representative of each group. **(B)** Panel A shows vimentin (green) strongly expressed around the microvasculature compared with GFAP (red) that was present around the periphery. However, panel B shows that both GFAP and vimentin co-localized in the astrocytes. Inset shows that GFAP is predominant in the astrocytic processes. Nuclei are stained in blue (DAPI).

Figure 8.

Proliferation is reduced in C3aR-inhibited mice. Proliferation (PCNA) was measured in MRL/*lpr* mice treated with vehicle (A) or C3aRa (B). Representative cortical cryosections stained with DAPI (blue) and PCNA (red) indicate that proliferation is reduced in MRL/*lpr* mice treated with C3aRa compared with those given vehicle alone.

Table 1

Primers used in quantitative reverse transcriptase polymerase chain reaction

<i>Genes</i>	<i>Primer 1 (Forward)</i>	<i>Primer 2 (Reverse)</i>
<i>C3aR</i>	5'-taaccagatgagcaccacca-3'	5'-tgtgaatgttgtgcatgg-3'
<i>MIP-2</i>	5'-caccaaccaccaggctac-3'	5'-gcccttgagagtggctatga-3'
<i>ICAM-1</i>	5'-cgcaagtccaattcacactga-3'	5'-cagagcggcagagcaaaag-3'
<i>TNF-α</i>	5'-ccgatgggtgtacctgtc-3'	5'-gtgggtgaggagcacgtagt-3'
<i>IFN-γ</i>	5'-actggcaaaaggatgggtgac-3'	5'-tgagctcattgaatccttg-3'
<i>GAPDH</i>	5'-gcaaattcaacggcacagt-3'	5'-agatggtgatggccttccc-3'