Complement component anaphylatoxins upregulate chemokine expression by human astrocytes

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Abstract The complement (C) system, a major component of the innate immune system, has been described as a factor implicated in some brain disorders. C activation leads to the release of anaphylatoxins, two proinflammatory polypeptides acting through specific receptors that have been detected on brain cells. Here, we examined the effect of anaphylatoxins on chemokine expression by human astrocytes. We showed that anaphylatoxins significantly increase chemokine mRNA expression. However, anaphylatoxin-induced chemokine secretion (interleukin-8) was observed only in the presence of interleukin-1β. Thus, anaphylatoxins could initiate a chemokine cascade and, at least in part, be involved in pathogenesis of the brain.

Key words: Complement; Brain; Astrocyte; Anaphylatoxin; Chemokine

1. Introduction

The complement (C) system is a key component of the innate immune system playing a central role in host defence against pathogens and in the initiation of inflammation [1]. Within the brain, C has been suspected to be implicated in the exacerbation of numerous human neurodegenerative disorders including Alzheimer's disease (AD) [2,3] and Pick's disease and demyelinating diseases such as multiple sclerosis (MS) [4]. Indeed, studies have demonstrated the lethal effects of C on brain cells in vitro and in experimental models of MS by lysing neurons and oligodendrocytes, respectively [5–7].

During inflammation, C activation leads to the release of various fragments including anaphylatoxins C3a and C5a liberated by the cleavage of C3 and C5 by C convertases. These potent proinflammatory peptides share several biological activities including mast cell degranulation, enhancement of vasodilatation, smooth muscle contraction and recruitment of immune cells to the site of inflammation [1]. They exert their biological activities by binding to a specific membrane receptor coupled to G proteins named C3aR and C5aR, respectively. Broadly distributed especially on myeloid cells, they have been shown to induce the production of some cytokines when stimulated [8,9]. In the central nervous system (CNS), glial cells and neurons express the C3aR and C5aR receptors [10,11] and our laboratory has contributed to the study of the role of anaphylatoxins in the CNS, especially on astrocytes [10–13]. We speculate that the C system may initiate, through anaphylatoxin release, a chemokine cascade.

Chemokines represent a large family of small, basic, soluble proteins that govern leukocyte transendothelial migration in a gradient-dependent manner. They and their receptors are considered the principal factor that drives the composition of inflammatory infiltrates by attracting and activating leukocytes. However, chemokines can inappropriately activate immune cells leading to inflammation and host cell destruction. In the CNS, chemokines are induced in various human diseases including AD [14], MS [15–17], human immunodeficiency virus encephalitis [18], and bacterial meningitis [19], and are thought to play an important role in pathogenesis.

The roles of anaphylatoxins on brain cells remain largely unknown and in order to evaluate their actions in the CNS, in the present study we studied the effect of C3a and C5a on chemokine expression by astrocytes. These new observations may have important implications for understanding of diseases and so far C3aR and C5aR could be considered new therapeutic targets.

2. Materials and methods

2.1. Reagents, cytokines and antibodies

Pertussis toxin (PTX), human recombinant C5a and interleukin (IL)-1β were purchased from Sigma (St Quentin Fallavier, France). Anti-C3a monoclonal antibody (G10) and anti-C5aR polyclonal antibody, used to block the effect of C3a and C5aR, respectively, were characterized previously [13,20]. Human C3a was generated by activation of C and purified as previously described [13]. Multiple-associated-peptide (MAP)-C3a and MAP-C5a peptides corresponding to the C-terminal part of the anaphylatoxins (amino acids correspond to 64–77 for C3a and 61–74 for C5a) attached to a poly-lysine comb (eight peptide monomers) were synthesized by solid phase synthesis (Applied Biosystems) and were purified by reverse phase high performance liquid chromatography. Sequences were ascertained by amino acid analysis.

2.2. Cell culture

The human glioblastoma cell line T98G was obtained from the

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Abbreviations: AD, Alzheimer's disease; C, complement; CNS, central nervous system; C3aR, C3a receptor; C5aR, C5a receptor; IL, interleukin; IP-10, interferon-γ-inducible protein of 10 kDa; Ltn, lymphotactin; MAP, multiple-associated-peptide; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MS, multiple sclerosis; PTX, pertussis toxin; RPA, ribonuclease protection assay; RANTES, regulated on activation, normal T cells expressed and secreted; TNF-α, tumor necrosis factor α

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American Type Culture Collection (Rockville, MD, USA). These cells were screened routinely by the Mycoplasma Detection Kit (Boehringer Mannheim, Meylan, France) to ensure that they were mycoplasma-free. Cells were grown in Ham's F12 culture medium (Biowhittaker, Emerainville, France) supplemented with 1% penicillin and streptomycin (Life Technologies, Cergy-Pontoise, France), and 10% heat-inactivated fetal calf serum (Life Technologies). Primary cultures of human fetal astrocytes were established from fetal brain (18-week-old fetus) as previously described [12]. The astrocyte marker glial fibrillary acidic protein was detected by flow cytometry in 95–97% of total cells. Experiments were conducted on cell cultures from the first to the third passage.

2.3. RNA extraction

Total RNAs were extracted from cells by the guanidinium isothiocyanate method followed by ultracentrifugation onto a cesium chloride cushion as described by Sambrook et al. [21]. Total RNAs (50 μg) were treated for 20 min at 37°C with 90 U of RQ-1 RNase-free DNase (Promega, Charbonnières, France) in 100 μl of buffer (40 mM Tris–HCl pH 8, 10 mM NaCl, 6 mM $MgCl_2$, 10 mM $CaCl_2$) and 200 U of RNasin ribonuclease inhibitor (Promega) to remove all traces of contaminating genomic DNA.

2.4. PCR primers

Human IL-8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were chosen according to their cDNA sequences reported in the EMBL data library under accession numbers XM031289 and M33197. Their sequences from 5′ to 3′ are: IL-8 (sense): TCT TGG CAG CCT TCC TGA TT; IL-8 (antisense): AAC TTC TCC ACA ACC CTC TG; GAPDH (sense): TGC CAT CAA CGA CCC CTT CA; GAPDH (antisense): TGA CCT TGC CCA CAG CCT TG. The theoretical size was 249 bp for IL-8 and 549 bp for GAPDH.

2.5. RT-PCR

Semi-quantitative RT-PCR was carried out with a GAPDH:IL-8 primer ratio of 1:75 and 1 μCi [^{33}P]dATP (Redivue, Amersham, Les Ulis, France) [20]. Experiments were conducted in which RNA bands after amplification were detected within the linear part of the amplifying curves so that plateau phases were not reached. Autoradiograms were analyzed by scanning using a Lecphor image analyzer (Biocom, Les Ulis, France). Results are expressed as the ratio of the area of the band of IL-8 to the mean of the area of the housekeeping gene band. The IL-8 mRNA value, from unstimulated cells, was arbitrarily set at one unit and values for the other samples were calculated relative to it.

2.6. Ribonuclease protection assay (RPA)

After total RNA extraction, RPA was performed using the RiboQuant Multiprobe RNase Protection Assay System (BD Pharmingen, Le Pont de Claix, France), according to the manufacturer's instructions. Briefly, the provided human chemokine template set (hCK-5) contained probes for eight chemokines: lymphotactin (Ltn), regulated on activation, normal T cells expressed and secreted (RANTES), interferon-γ-inducible protein of 10 kDa (IP-10), macrophage inflammatory protein 1α and 1β (MIP-1α, MIP-1β), monocyte chemoattractant protein (MCP-1), IL-8, and I-309, and two housekeeping genes (GAPDH and L-32). To synthesize antisense cRNA, the probes were labeled with [^{32}P]αUTP (800 Ci/mmol, 10 mCi/ml; Amersham) using a transcription kit according to the manufacturer's manual. Ten micrograms of each sample was used for hybridization with the antisense RNA probe at 56°C for 12–16 h, followed by digestion of free probe and unprotected single-stranded RNA with RNase solution (RNase A plus RNase T1). The remaining double-stranded RNA was then extracted in chloroform/isoamyl alcohol (50:1) and was precipitated with ethanol and separated on a 7 M urea/6% polyacrylamide gel. A part of the undigested probe was used as a marker standard. After drying, the gel was placed in the exposure cassette with a phosphor screen for 48 h. Bands were detected by phosphorimaging using ImageQuant software (Molecular Dynamics). A standard curve plotted with the undigested probe markers was used to identify the bands of various genes in the experimental samples. Results were expressed as the ratio of the volume of the band of interest to the mean of the volumes of the bands for the housekeeping genes. The chemokine mRNA value, from unstimulated cells, was arbitrarily set at one unit and values for the other samples were calculated relative to it.

2.7. Enzyme-linked immunosorbent assay (ELISA)

Concentrations of IL-8 protein in culture supernatant samples were determined by a specific sensitive ELISA as previously described [22]. The absorbance was measured at A_{492nm} with a plate reader (Labsystems iEMS Reader MF) and IL-8 concentration in the samples was determined by comparison with an IL-8 standard curve. The level of IL-8 in the culture supernatants was expressed as pg/ml per 10^6 cells.

2.8. Statistical analysis

Data are expressed as the mean ± S.E.M. Statistical analysis was performed using Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. C3a and C5a anaphylatoxins enhanced chemokine mRNA expression on human glioblastoma cells

In a first approach, we studied chemokine mRNA expression on the human glioblastoma cell line T98G. This cell line expressed C3a and C5a receptors [12,23] and responded to anaphylatoxin stimulation [20].

T98G cells were stimulated by MAP-C3a or MAP-C5a (10^{-8} M), two strong peptide anaphylatoxin analogues, and total RNAs were extracted after different stimulation times (2, 4, 6, 12 and 24 h). The variation of chemokine mRNA expression was analyzed by the sensitive RPA technique. Thus,

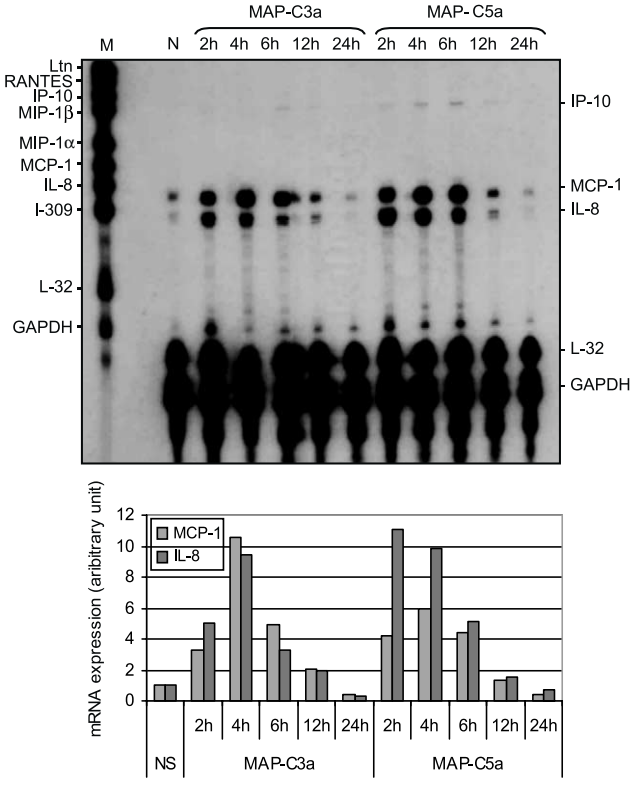

Fig. 1. Expression of chemokines by T98G cells stimulated by MAP-C3a or MAP-C5a (10^{-8} M). T98G cells were stimulated for 2, 4, 6, 12 or 24 h by MAP peptides and chemokine mRNA expression was determined by RPA. A representative blot is shown in the top panel and the matching ratios expressed as relative fold increase over control are represented in the bottom panel. Lane M represents the multi-probe template set not treated with RNases which serves as a ladder. NS = non-stimulated cells.

at the same time, we studied Ltn, RANTES, IP-10, MIP-1α, MIP-1β, IL-8 and I-309 mRNA expression using GAPDH and L-32 as internal standards. Results are presented as relative fold increase over control (unstimulated cells). We observed that unstimulated T98G cells constitutively express low levels of MCP-1 and IL-8 mRNA (Fig. 1, top).

Stimulation of T98G cells induced a high increase of MCP-1 mRNA expression which was increased 10.5-fold and 6.0-fold after 4 h stimulation with MAP-C3a and MAP-C5a, respectively (Fig. 1, bottom). The IL-8 mRNA level was also increased 9.4-fold after 4 h of stimulation by MAP-C3a and 11-fold after 2 h of stimulation by MAP-C5a. We also detected a weak induction of IP-10 mRNA expression, especially after MAP-C5a stimulation. However, MAP peptide stimulation did not induce Ltn, RANTES, MIP-1α, MIP-1β or I-309 mRNA on T98G cells.

The upregulation of IL-8 mRNA expression induced by MAP-C3a and MAP-C5a was also observed when using human purified C3a and recombinant C5a. T98G cells were stimulated with C3a or C5a (10^{-8} M) and IL-8 mRNA expression was studied by semi-quantitative RT-PCR. After 4 h of stimulation, the IL-8 mRNA level was increased by 5.3-fold and 6.2-fold, respectively (Fig. 2).

3.2. *Anaphylatoxin-upregulated IL-8 mRNA expression was specific and dose-dependent*

In order to ascertain the specificity of anaphylatoxins in the upregulation of IL-8 mRNA, we tried to block their effect by specific antibodies. Pre-incubation of C3a anaphylatoxin with an anti-C3a monoclonal antibody (1/100) for 30 min completely blocked the upregulation of IL-8 gene expression induced by C3a alone (Fig. 2). Similarly, when cells were pre-incubated for 30 min with an anti-C5aR polyclonal antibody (1/100) before C5a stimulation, the C5a-induced IL-8 mRNA upregulation was abolished (Fig. 2).

C3a and C5a anaphylatoxins are known to bind to their own receptors, which are functionally coupled to G proteins [24,25]. To confirm that anaphylatoxin stimulation leading to the upregulation of IL-8 mRNA acted through a G protein-coupled pathway, T98G cells were pre-incubated with PTX

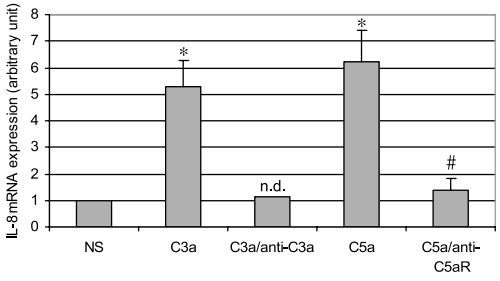

Fig. 2. Expression of IL-8 mRNA after stimulation of T98G cells by C3a or C5a anaphylatoxins (10^{-8} M). Effect of pre-incubation with anti-C3a or anti-C5aR antibodies. C3a anaphylatoxin was pre-incubated for 30 min with an anti-C3a antibody (diluted 1/100), or cells were pre-incubated for 30 min with an anti-C5aR antibody (diluted 1/100) before 4 h stimulation by C3a or C5a (10^{-8} M), respectively. Total RNAs were extracted and RT-PCR was performed. Bars represent the mean ± S.E.M. of triplicate samples. A representative graph of $n = 3$ independent experiments is shown. $^{*}P < 0.05$, statistically significant difference compared with non-stimulated cells (NS) as determined by Student's t-test; $^{\#}P < 0.05$, statistically significant difference compared with cells stimulated with C5a as determined by Student's t-test; n.d., not determined.

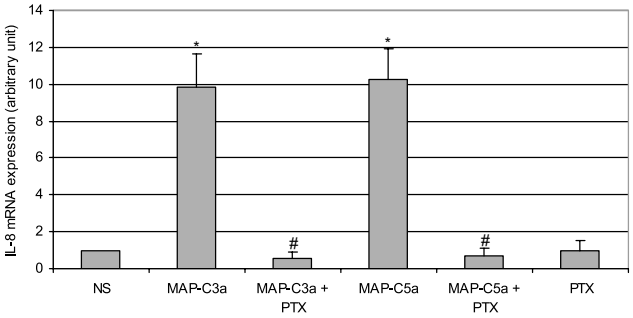

Fig. 3. Influence of PTX on the IL-8 mRNA production by T98G cells following stimulation with MAP-C3a or MAP-C5a (10^{-8} M). Cells were pre-incubated for 4 h with PTX (200 ng/ml) and then stimulated by MAP-C3a or MAP-C5a for 4 h. Cells were also incubated with PTX alone. Total RNAs were extracted and RT-PCR was performed. Bars represent the mean ± S.E.M. of triplicate samples. A representative graph of $n = 3$ independent experiments is shown. $^{*}P < 0.05$, statistically significant difference compared with non-stimulated cells (NS) as determined by Student's t-test; $^{\#}P < 0.05$, statistically significant difference compared with cells stimulated with MAP peptides as determined by Student's t-test.

(200 ng/ml) for 4 h before application of MAP peptides. After 4 h of stimulation by anaphylatoxin agonists or without stimulation, the level of IL-8 mRNA was determined. PTX alone had no effect on IL-8 expression but pre-treatment of cells by PTX completely abrogated the upregulation of IL-8 mRNA expression induced by anaphylatoxin agonists (Fig. 3).

T98G cells were stimulated with increasing concentrations of MAP peptides or anaphylatoxins in a concentration range of 10^{-13}–10^{-7} M. Total RNAs were extracted after 4 h of stimulation and IL-8 mRNA expression was measured. From 10^{-10} to 10^{-8} M, we observed a linear relationship between IL-8 mRNA expression and the concentration of different peptides showing a dose-dependent increase of IL-8 mRNA (data not shown).

3.3. *C3a and C5a anaphylatoxins enhanced chemokine mRNA expression on human fetal astrocytes*

To confirm previous results obtained with the T98G cell line, we examined chemokine expression on human primary astrocytes stimulated by MAP-C3a or MAP-C5a (10^{-8} M). The variation of chemokine mRNA expression after different stimulation times (2, 4, 6, 12 and 24 h) was carried out using the RPA technique. Results representing relative fold increase over control (unstimulated cells) are shown in Fig. 4.

Thus, we observed that human fetal astrocytes constitutively expressed low level of RANTES, IP-10, MCP-1 and IL-8 but not Ltn, MIP-1α, MIP-1β or I-309 mRNA. Stimulation of astrocytes by MAP peptides increased the RANTES, IP-10 and MCP-1 mRNA expression two-fold at most. As for glioblastoma cells, IL-8 mRNA expression exhibited the greatest increase after 4 h of peptide stimulation. We also detected an induction of MIP-1α mRNA expression.

3.4. *C3a and C5a stimulation increased IL-8 release by T98G cells*

As IL-8 mRNA expression showed the higher increase in response to anaphylatoxin stimulation, we wanted to study IL-8 secretion by astrocytic cells stimulated with anaphylatoxins. The supernatants of stimulated T98G cells were collected after 12, 24, 48 and 72 h stimulation by either MAP peptides

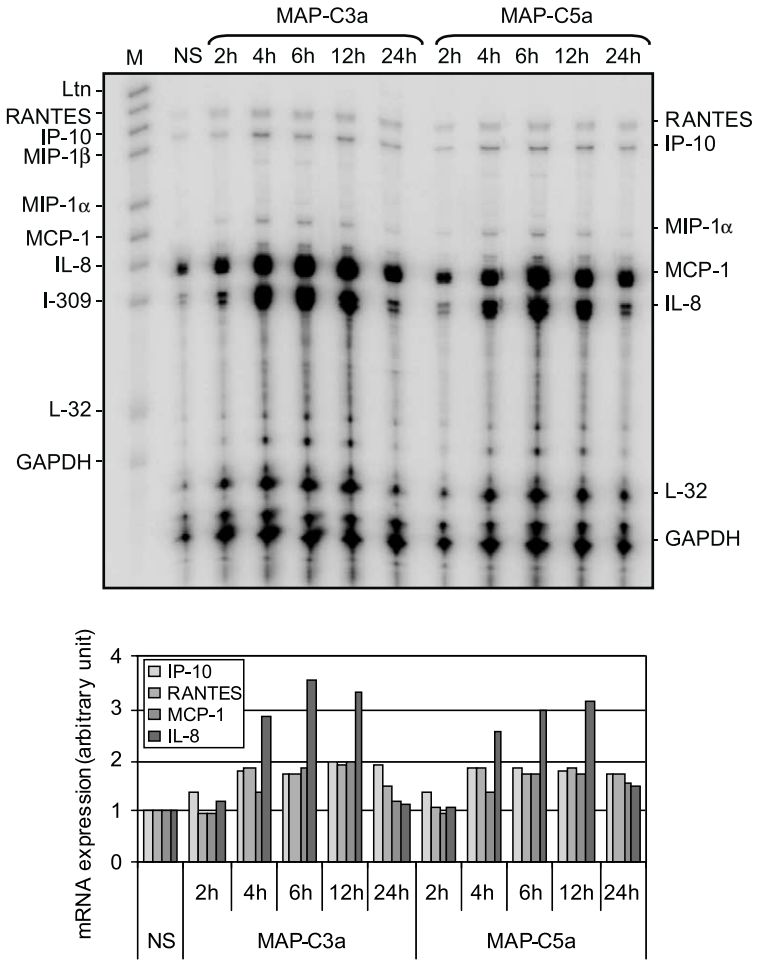

Fig. 4. Expression of chemokines by fetal astrocytes stimulated by MAP-C3a or MAP-C5a (10^{-8} M). A representative autoradiogram of chemokine mRNA expression by fetal astrocytes after 2, 4, 6, 12 or 24 h of stimulation by MAP peptides is shown in the top panel. See legend of the Fig. 1.

or anaphylatoxins (10^{-8} M) and the concentration of IL-8 protein was analyzed by specific sensitive ELISA. The release of IL-8 is time-dependent in response to C3a/C5a and was maximal after 48 h of stimulation (data not shown). The concentration of IL-8 was then analyzed by specific ELISA. The results are presented in Fig. 5.

A basal level of IL-8 produced by T98G cells was detected (54 ± 16 pg/ml/10^6 cells) and a weak increase of the IL-8 amount in the supernatant was observed after MAP peptide stimulation. After 48 h of stimulation, the IL-8 amount reached 144 ± 32 pg/ml/10^6 cells for MAP-C3a and 83 ± 24 pg/ml/10^6 cells for MAP-C5a. No significant increase of IL-8 secretion was observed after anaphylatoxin stimulation.

As anaphylatoxins are generated in an inflammatory context where other cytokines, including IL-1β, are also released, we wanted to investigate the effect of anaphylatoxin/IL-1β co-stimulation on IL-8 release. First, we measured the release of IL-8 in the supernatant of T98G cells after 48 h of stimulation by increasing concentrations of IL-1β in order to determine the sub-optimal dose of IL-1β which did not increase the IL-8 secretion rate (Fig. 6A).

No increase of IL-8 secretion was observed for concentrations of IL-1β below 0.05 U/ml. Then, we co-stimulated T98G cells for 48 h with IL-1β (0.03 U/ml) and with C3a, C5a, MAP-C3a or MAP-C5a (10^{-8} M) (Fig. 6B). We observed that when T98G cells were co-stimulated by anaphylatoxins (10^{-8} M) and IL-1β (0.03 U/ml), the IL-8 release was highly increased compared to that after stimulation by anaphylatoxins alone. Thus, IL-8 secretion reached 239 ± 57 pg/ml/10^6 cells for IL-1β 0.03 U/ml+C3a, 300 ± 61 pg/ml/10^6 cells for IL-1β 0.03 U/ml+C5a, 478 ± 69 pg/ml/10^6 cells for IL-1β 0.03 U/ml+MAP-C3a and 383 ± 69 pg/ml/10^6 cells for IL-1β 0.03 U/ml+MAP-C5a showing a synergistic effect of anaphylatoxins and IL-1β on the secretion by astrocyte cells.

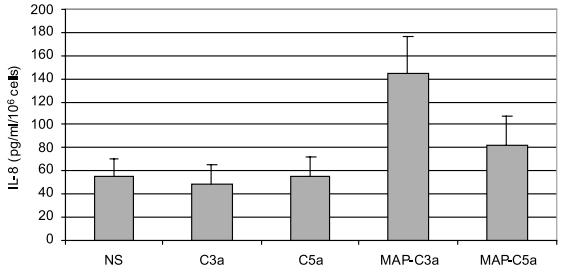

Fig. 5. Production of IL-8 by T98G cells by ELISA after incubation with C3a, C5a, MAP-C3a or MAP-C5a (10^{-8} M) for 48 h. The concentration of IL-8 was measured in the supernatants and is expressed as pg/ml per 10^6 cells. Bars represent the mean ± S.E.M. of triplicate samples. A representative graph of $n = 3$ independent experiments is shown.

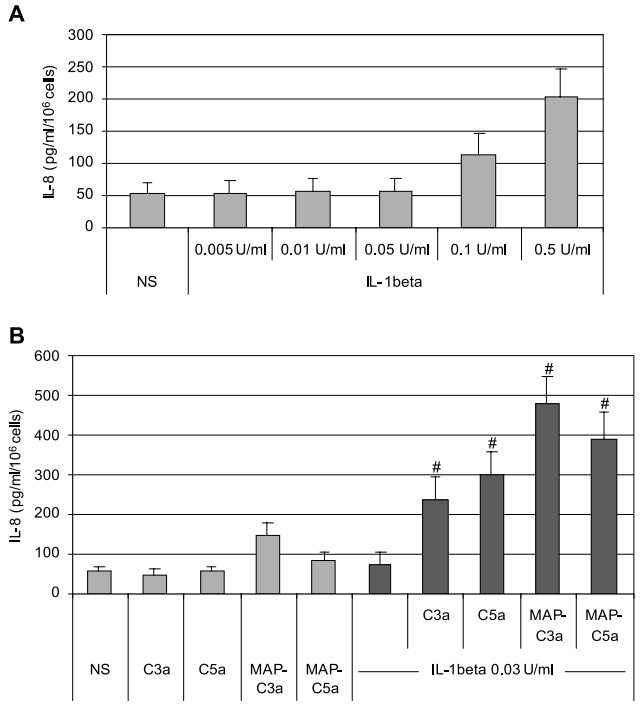

Fig. 6. Effect of anaphylatoxin/IL-1β co-stimulation on IL-8 release by T98G cell line. First, cells were stimulated for 48 h by increasing doses of IL-1β to determine the maximal concentration of IL-1β which did not induce IL-8 release (A). Second, cells were co-stimulated for 48 h with a sub-optimal dose of IL-1β (0.03 U/ml) with C3a, C5a, MAP-C3a or MAP-C5a (10^{-8} M) (B). The concentration of IL-8 was measured in the supernatants by ELISA and is expressed as pg/ml per 10^6 cells. Bars represent the mean ± S.E.M. of triplicate samples. A representative graph of $n = 3$ independent experiments is shown. $^{\#}P < 0.05$, statistically significant increase compared with cells stimulated with IL-1β 0.03 U/ml as determined by Student's *t*-test.

4. Discussion

The C system plays an important role in the control of infection by lysis and opsonization of microorganisms and in the initiation and the control of inflammation. C3a and C5a anaphylatoxins, generated during C activation by the proteolytic cleavage of C3 and C5 components, are two strong inflammatory polypeptides. C5a and to a lesser extent C3a are important chemoattractant molecules that have the capacity to modulate synthesis of cytokines such as IL-1β, tumor necrosis factor α (TNF-α), IL-6 and IL-8 by myeloid cells [8,9,26–29]. Anaphylatoxins regulate inflammatory functions by interacting with their own receptors, C3aR and C5aR, coupled to PTX-sensitive G protein [30–33]. Traditionally, C3aR and C5aR were thought to be present only on myeloid cells. However, further studies have demonstrated these receptors on non-myeloid cells and both receptors have been detected on neurons and glial cells of the CNS [10–12,23]. Interestingly, our laboratory has shown that astrocytes stimulated with anaphylatoxins produced increased levels of IL-6 mRNA while the levels of IL-1β, TNF-α and transforming growth factor-β remained unaffected [20]. The properties of anaphylatoxins in the CNS remain largely unknown and to obtain further clues on their actions, we examined the release of chemokines by astrocytes upon stimulation with anaphylatoxins using the sensitive RPA technique. The chemokines examined were RANTES, MIP-1α, MIP-1β, MCP-1 and I-309, belonging to the CC chemokine family, and IP-10 and IL-8, two CXC chemokines.

First, we investigated chemokine mRNA expression in response to anaphylatoxins on the human T98G cell. We observed that this cell line expressed constitutively low levels of MCP-1 and IL-8 mRNA and we showed an increased expression of them and an induction of IP-10 mRNA expression after stimulation by anaphylatoxins. The anaphylatoxin-induced IL-8 release has been shown to be dose-dependent since IL-8 mRNA expression varied with the concentration of anaphylatoxins and to act through a G protein-coupled pathway since pre-treatment of cells with PTX completely blocked the IL-8 response. Furthermore, pre-incubation with anti-C3a or anti-C5aR antibodies abolished the C3a- and C5a-induced IL-8 mRNA increase respectively, showing that the response was specific.

Chemokine mRNA expression was also studied on fetal human primary astrocytes stimulated by anaphylatoxins. Unstimulated cells expressed low levels of RANTES, IP-10, MCP-1 and IL-8 mRNA which increased after anaphylatoxin stimulation. We also observed an induction of MIP-1α mRNA. Astrocytes have already been shown to respond to several proinflammatory cytokines such as IL-1β, TNF-α or interferon-γ to induce some chemokine expression [34]. Thus, anaphylatoxins, released during C activation, could be another stimulus that leads to chemokine upregulation in the CNS.

Then, we investigated IL-8 secretion by T98G cells in response to anaphylatoxins. Cells were stimulated for 48 h with C3a, C5a or MAP peptides and the concentration of IL-8 was determined in the supernatant by specific ELISA assay. Thus, compared to unstimulated cells, we observed a weak increase of IL-8 secretion only after stimulation by MAP peptides. This phenomenon of transcription activity without protein synthesis has already been observed by several groups in different cell types. Peripheral blood mononuclear cells stimulated by C5a anaphylatoxin have shown significant levels of IL-1β mRNA without the appearance of a proportional amount of IL-1β protein [27,35,36]. Our laboratory has also observed a high increase of IL-6 mRNA expression by astrocytes after anaphylatoxin stimulation without detecting any protein [20]. As anaphylatoxins are generated in an inflammatory context where cytokines are also released, we investigated the effect of anaphylatoxin/IL-1β co-stimulation on IL-8 release. We showed here an effect of a sub-optimal dose of IL-1β on the anaphylatoxin-induced IL-8 release. Thus, even if anaphylatoxins alone are unable to enhance chemokine secretion by astrocytes, they could when co-stimulated with proinflammatory cytokines such as IL-1β. As the C system could be inappropriately activated leading to the promotion of serious damage in the CNS, this mechanism may constitute a protection against inappropriate activation of the C system and stop the inflammatory cascade. Thus, anaphylatoxins could play an important role in priming astrocytes during inflammatory responses in the brain.

Our results demonstrated several homogeneous and specific patterns of chemokine gene expression by human astrocytes in response to anaphylatoxins. Chemokines seem to play a crucial role in neuroinflammation where most diseases are accompanied by chemokine expression, including MCP-1, MCP-2, MIP-1α, MIP-1β, RANTES, IL-8 and IP-10 [37,38]. Several studies have demonstrated the expression of

chemokines and especially IP-10 and RANTES in the CNS tissue of MS [17,39–43]. Because they are potent T-cell chemoattractants, their elevated levels during active episodes of MS may induce migration of T-cells into the brain. During AD, it is suggested that chemokine expression may also contribute to the development and/or the progression of the disease [14].

The role of chemokines in control of CNS bacterial infections is not well understood. However, it has been demonstrated that patients with bacterial meningitis show chemokine expression in the cerebrospinal fluid [44]. Furthermore, when mouse brains were infected by *Staphylococcus aureus*, chemokines were expressed locally [45]. These examples point out the involvement of chemokines in host defence against bacterial infections of the CNS.

Our data show that stimulation of human astrocytes by anaphylatoxins could lead to chemokine mRNA upregulation or induction and to possible chemokine secretion (IL-8) but only when co-stimulated with IL-1β. The most general response to chemokine stimulation is chemotaxis of different types of leukocytes. Thus, chemokine upregulation by astrocytes in response to C3a and C5a anaphylatoxins may have important implications during brain inflammatory diseases.

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