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Research article

Antibody/receptor protein immunocomplex in human and mouse cortical nerve endings amplifies complement-induced glutamate release

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HIGHLIGHTS

- The classic pathway of activation is not involved in the complement-evoked glutamate release.
- Complement glutamate release is significantly increased in synaptosomes incubated with anti-NH_2-CCR5 antibody.
- C1q deprived serum-induced glutamate release is unaltered in terminals incubated with anti-NH_2-CCR5 antibody.
- Complement glutamate release is unmodified in anti-COOH-CCR5 entrapped synaptosomes.
- Antibodies recognizing the outer sequence of receptors triggers the activation of complement trough the classic pathway.

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Abbreviations:
BBB, blood-brain barrier
RANTES, regulated upon activation normal T cells
expressed and secreted
CCRs, C–C chemokine receptors

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ABSTRACT

Previous studies have demonstrated that complement alone releases glutamate from human and mouse cortical terminals in an antibody-independent manner. In order to expand our knowledge on complement-mediated effects, we investigated whether the presence of an antigen-antibody complex in synaptosomal plasmamembranes could also trigger complement-induced functional responses that might affect neurotransmitter release. To this aim, we focused on the chemokine 5 receptor (CCR5) expressed in human and mouse cortical glutamate terminals, whose activation by CCL5 elicits [^{3}H]D-aspartate ([^{3}H]D-ASP) release. Preincubating synaptosomes with an antibody recognizing the NH_2 terminus of the CCR5 protein (anti-NH_2-CCR5 antibody) abolished the CCL5-induced [^{3}H]D-ASP release. Similarly, enriching synaptosomes with an antibody recognizing the COOH terminus of CCR5 (anti-COOH-CCR5 antibody) prevented the CCL5-induced [^{3}H]D-ASP release. The antagonist-like activity of the anti-NH_2-CCR5 antibody turned to facilitation when anti-NH_2-CCR5-treated synaptosomes were exposed to complement. In these terminals, the releasing effect was significantly higher than that elicited by complement in untreated synaptosomes. On the contrary, the complement-induced [^{3}H]D-ASP release from anti-COOH-CCR5 antibody-entrapped synaptosomes did not differ from that from untreated synaptosomes. Preincubating synaptosomes with anti-beta tubulin III antibody, used as negative control, neither prevented the CCL5-induced releasing effect nor it amplified the complement-induced [^{3}H]D-ASP release. Finally, serum lacking the C1q protein, i.e. the protein essential to promote the antibody-mediated activation of complement, elicited a comparable [^{3}H]D-ASP release from both untreated and anti-NH2-CCR5 antibody-treated synaptosomes. Thus, we propose that antibodies raised against the outer sequence of a receptor protein can trigger the activation of the complement through the classic, C1q-mediated antibody-dependent pathway, which results in an abnormal release of glutamate that could be deleterious to central nervous system.

1. Introduction

Complement is a complex network of soluble and membrane proteins that drives the humoral and cellular immune responses. Complement activation is proposed to occur through three differ-

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ent pathways. The first is the classic, antibody-dependent pathway, which represents the humoral response of complement to the presence of immunocomplexes with IgG1 and/or IgM antibodies. The second one is the lectin-dependent pathway, that is initiated by lectin recognizing sugar structures, while the third is the alternative pathway, which is triggered by the degradation of the C3 component of the complement system [1,2].

Very recently, complement was shown to release glutamate from glutamatergic nerve endings isolated from different central nervous system (CNS) regions, without affecting the release of other neurotransmitters (i.e. noradrenaline, acetylcholine or GABA as well, [3]). The pathway of complement activation involved in the releasing effect has not so far been investigated. However, since the presence of antibody/receptor protein complexes in synaptosomal plasmamembranes could not in principle be excluded, we have investigated the possibility that the classic pathway could be involved. Two different approaches were used to address this question.

First, synaptosomes were incubated with an antibody recognizing the outer sequence of receptor proteins known to be present in the plasmamembranes of glutamatergic nerve endings. This allowed us to determine whether the presence of an antibody/receptor protein complex in synaptosomal plasmamembranes could affect the complement-induced releasing efficiency in these terminals. To this aim, we focused on the chemokine receptor subtype 5 (CCR5). Beside their well-known expression in macrophages and T-lymphocytes, CCR5s are also widely distributed in non-immunocompetent cells. In particular, as far as the CNS is concerned, CCR5 exists in glutamate neurones as well as in astrocytes where its activation controls glutamate release [4–6]. Taking into consideration that the natural ligand of CCR5, the chemokine CCL5 (RANTES, regulated upon activation normal T cells expressed and secreted), is actively produced and secreted by glial cells, the CCL5-induced glutamate release could represent a mechanism of glial to neuron communication, which is crucial to neuronal plasticity and developmental processes, and potentially relevant in neuroinflammatory diseases [7,8]. In recent years we showed that antibodies recognizing the outer sequence of the CCR5 receptor protein (anti-NH_2-CCR5-antibody) can abolish the chemokine-mediated glutamate release possibly by impeding the binding of the natural ligand CCL5 at CCR5 then [6,9]. Synaptosomes bearing anti-NH_2-CCR5-antibody/CCR5 protein complexes were exposed in superfusion to complement, in order to assess whether the presence of the immunocomplex could elicit unexpected functional responses that could emerge as changes in glutamate outflow. The complement-induced releasing effect in anti-NH_2-CCR5-antibody pretreated synaptosomes was significantly higher than that from untreated synaptosomes, suggesting that other releasing mechanism(s) took place because of the presence of the antibody/receptor protein complexes.

As a second approach, we directly verified the involvement of the classic pathway of activation of complement by analysing the impact of C1q deprived serum in human and mouse cortical glutamatergic synaptosomes. The C1q component is essential to trigger the complement activation through the classic pathway, so that the use of the deprived serum could allow us to investigate the involvement of this pathway in the complement-induced releasing effects. Interestingly, the anti-NH_2-CCR5-antibody-mediated complement-induced releasing effect was not observed in synaptosomes exposed to the C1q-deprived serum. This observation is compatible with the idea that the classic pathway of activation of complement had a role in eliciting the antibody-mediated releasing effect.

2. Materials and methods

2.1. Tissue samples and synaptosomes preparation

Samples of human cerebral cortex (parts of frontal and temporal lobes) were obtained from informed and consenting patients (7 women, 4 men, aged 27-58 years, for premedication and anaesthesia [6,10]) undergoing neurosurgery to reach deeply seated tumours. Adult male mice (C57BL/6, 20–25 g, Charles River, Calco, Italy) were killed by decapitation and the cortices were rapidly removed. The animal experimental procedures were in accordance with the European legislation (European Communities Council Directive of September, 22, 2010, n° 2010/63/EU) and the Italian legislation (L.D. no. 116 /1992 and 26/2014), and they were approved by the Italian Ministry of Health (protocol number n° 29,823-10). Tissues were placed in a physiological salt solution at 2–4 °C and synaptosomes were prepared within 30 min as previously described [7].

2.2. Release experiments

Purified synaptosomes were resuspended in a physiological solution having the following composition (mM): NaCl, 140; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 5; HEPES, 10; glucose, 10; pH 7.2–7.4 and incubated at 37 °C, for 15 min in the presence of [2,3-^{3}H]D-aspartate ([^{3}H]D-ASP, specific activity 11.3Ci/mmol, f.c. 50 nM, Perkin Elmer, Boston, MA), an unmetabolizable analogue of glutamate often used in release studies to monitor glutamate release [11]. Synaptosomes were pre-incubated with mouse anti-CCR5 antibody raised against the NH_2 terminus of the CCR5 receptor protein (CCR5-03,IgM, recognising the 13–28 amino acid sequence of the human CCR5 receptor, NIBSC, Centralized Facility for AIDS reagents, Hertfordshire, UK, the anti-NH_2-CCR5 antibody in the text, 1:2000) or with mouse anti-β-tubulin III (Sigma–Aldrich, Milan, Italy, 1:400), used as negative control. In control experiments, synaptosomes were incubated with a rabbit anti-CCR1 antibody recognizing the NH_2 terminus of the CCR1 receptor protein (rabbit anti-CCr1, IgG, Novus Biological, Cambridge, UK, the anti-NH_2-CCR1 antibody in the text 1:2000). When indicated, rabbit anti-CCR5 antibody recognizing the COOH terminus of the receptor (CKR-5, IgG, ANASPEC, S. Jose, CA, the anti-COOH-CCR5 antibody throughout the text, 1:400) were entrapped within synaptosomes by homogenizing human tissue specimens with sucrose containing the antibody [6,10]. Based on estimates made by entrapping [^{3}H]sucrose, the intrasynaptosomal concentration of the antibody in the cytosol should amount to about the 5% of the original concentration in the homogenization medium. Identical portions of the synaptosomal suspensions were layered at the bottom of superfusion chambers in a Superfusion Apparatus (Ugo Basile, Comerio, Varese, Italy, for technical details see Ref. [12]) at 37 °C and were superfused for 36 min to equilibrate the system. Three consecutive 3-min fractions (termed b1 to b3) were collected and counted for radioactivity. Human and mouse (lyophilized, Low Tox) complement (Cederlane, Ontario, Canada), complement C1q-depleted human serum (Calbiochem, EMD Millipore Inc., Billerica, MA, USA) or CCL5 (Sigma–Aldrich, Milan, Italy) were introduced at the end of the first fraction collected and then replaced with standard medium after 3 min of superfusion. Superfusate fractions and synaptosomes were then counted for radioactive content with liquid scintillation.

2.3. Calculations and statistics

Fractions collected and superfused synaptosomes were counted for radioactivity that was expressed as a percentage of the total