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BRAIN RESEARCH

Research Report

Role of complement component C5 in cerebral ischemia/reperfusion injury

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C1-INH, C1-inhibitor
CVF, cobra venom factor
MCAO, middle cerebral artery occlusion
sCR1, soluble complement receptor 1
ZAS, zymosan-activated rat serum

ABSTRACT

We evaluated the role of complement component C5 during the course of cerebral ischemic reperfusion injury in a rat model of middle cerebral artery occlusion (MCAO). Systemic C5 inhibition was achieved with an anti-C5 monoclonal antibody, which significantly prevented the deterioration of the motor functions by reducing cerebral lesion and edema. Our results show that activated C5 complement components played an important role in cerebral tissue inflammation resulting from ischemia/reperfusion injury.

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1. Introduction

Cerebral stroke continues to be a leading cause of death and disability worldwide due to the limited efficacy of current therapy (Fisher, 2002). There is a great need for curative treatments, but also for good prevention protocols for populations at high stroke risk (Wolf et al., 1999). A better understanding of the pathogenesis of cerebral tissue inflammation, which occurs after an ischemia/reperfusion insult, may contribute to the development of new prophylactic and

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therapeutic treatments. The complement system is a key component of innate immunity that plays a major role in host defense. However, some of the activated components of the complement cascade are also known to be potent proinflammatory mediators and have been reported to participate in ischemia/reperfusion injury (Bhole and Stahl, 2003; Seelen et al., 2005). Complement can be activated through the classical, the alternative and the lectin pathways (Seelen et al., 2005). The classical and lectin pathways are initiated by the deposition of antibody and serum lectins such as mannose-binding lectin (MBL), respectively. Both generate membrane-bound C4b, C3b and the complex C5b-9 in this order, as well as producing anaphylatoxins C4a, C3a and C5a. The alternative pathway starts by the direct deposition of C3b, which initiates a proteolytic cascade that converges with the other complement pathways at the C5 cleavage (Seelen et al., 2005). C3b is also known for its ability to opsonize immune complexes and microbials (Carroll, 1998). In fact, the components C1q, C4b, C3b and breakdown products are ligands for several complement receptors expressed on immune cells and promote immune functions such as B cell activation and phagocytosis (Carroll, 1998; Seelen et al., 2005). Lastly, C5a recruits and activates leukocytes, whereas the membrane-attack complex (C5b-9) leads to cell lysis and death (Seelen et al., 2005). Thus, all three pathways involve the activation of C3 and C5 complement components, which are considered to be attractive targets for blocking complement activation (Bhole and Stahl, 2003).

Recent studies have pointed out a role of complement activation in cerebral ischemia/reperfusion injury. Focal deposition of C5b-9 has been detected in the brain infarcted areas of stroke patients (Lindsberg et al., 1996). Furthermore, transitory systemic complement activation follows stroke in humans (Pedersen et al., 2004). However, it is not well understood how activated complement components contribute to cerebral tissue inflammation during ischemia/reperfusion injury and what is the role of the different complement proteins. Complement inhibition has shown success in several small animal models of cerebral ischemia (Huang et al., 1999; Heimann et al., 1999; De Simoni et al., 2003; Akita et al., 2003). The use of soluble complement receptor 1 (sCR1) led to a reduction in infarct volume and neutrophil accumulation after 45-min MCAO in mice (Huang et al., 1999). A neuroprotective effect of C1-inhibitor (C1-INH) was also observed in various rodent models of focal cerebral ischemia (Heimann et al., 1999; De Simoni et al., 2003; Akita et al., 2003). Akita et al. (2003) suggested that this effect is due to diminished recruitment of inflammatory cells to the ischemic area. Finally, cobra venom factor (CVF) administered 24 h prior to MCAO also reduced dramatically cerebral infarct volume in rats (Figueroa et al., 2005). Thus, these studies support a role of complement activation in brain infarction. Nevertheless, these early studies used reagents that were not specific inhibitors of a single complement component and may potentially compromise the opsonizing and host defense functions of the complement cascade. The complement receptor 1 and its soluble form sCR1 bind different components of the complement cascade including C1q, MBL, C4b and C3b (Liszewski and Atkinson, 1993; Huang et al., 1999; Ghiran et al., 2000). C1-INH also inhibits components from the three complement pathways (C1r, C1s, MBL-associated serine proteinases-1 and -2, C3b), in addition to proteases of the contact system of kinin generation, coagulation factors and selectins (Carroll, 1998; Jiang et al., 2001; Cai and Davis, 2003). Finally, CVF depletes all complement components downstream of C3 (Figueroa et al., 2005).

We have hypothesized that activated complement components, in particular C5a and C5b-9, are critically involved in the development of cerebral inflammation during ischemia/reperfusion injury. In this study, we set up to elucidate the role of activated C5 components in a rat MCAO model. To this end, we used a monoclonal antibody specifically blocking the cleavage of C5 into C5a and C5b-9, which prevents the generation of these potent proinflammatory mediators. The impact of systemic C5 inhibition on the development of cerebral inflammation after MCAO is presented and discussed.

2. Results

2.1. *Systemic inhibition of complement component C5*

To assess the role of C5 in a rat MCAO model, we used the anti-rat C5 mAb 18A10.62, which prevents C5 cleavage and the generation of C5a and C5b-9 (Vakeva et al., 1998). To further characterize the antibody function, we evaluated the ability of 18A10.62 to bind directly to either C5b or C5a once C5 is cleaved. We observed that 18A10.62 did not bind C5b after inducing complement activation on ELISA plates (another anti-C5 antibody 13E8.15 did, data not shown). Moreover, it did not inhibit C5a directly either. This effect was determined in vitro in a functional assay that measured the ability of 18A10.62 to prevent C5a-induced migration of rat neutrophils after exposure to zymosan-activated rat serum (Fig. 1A). The anti-C5 mAb 18A10.62 effectively averted neutrophil migration when added to the serum prior to zymosan, but did not inhibit it when added afterwards (Fig. 1A).

The 18A10.62 dosing regime (described in the Experimental Procedure) was established following pharmacodynamic experiments in male Wistar rats. We based our anti-C5 mAb treatment on the presented pharmacodynamic experiment (Fig. 1B), which comprised an i.v. injection of 20 mg/kg 18A10.62 at time 0 and 2 i.p. injections (10 mg/kg) given 6 and 25 h later. This protocol attained more than 50% reduction in serum C5 activity as assessed by C5b-9-mediated hemolytic assay. We determined a 60% inhibition at 2 h and 12 h after initial i.v. administration of 18A10.62 and around half the activity at the other time points assessed. In the MCAO experiments, C5 inhibition was started at two different time points relative to the initiation of MCAO: 1 h prior to MCAO (pretreatment experiment) and 5 min after (posttreatment experiment). Note that we did not bleed the rats subjected to MCAO during the study, but we conducted a parallel treatment in healthy rats and determined serum C5 activity daily that confirmed comparable C5 inhibition (data not shown).

2.2. *Changes of physiological parameters after MCAO*

During the MCAO experiments, we monitored changes in body weight and rectal temperature and observed no

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