Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 215 (2010) 395–405
Immunobiology
www.elsevier.de/imbio

Specific inhibition of the classical complement pathway with an engineered single-chain Fv to C1q globular heads decreases complement activation by apoptotic cells

Marcus R. Duvall[a], Hee Young Hwang[a], Robert J. Boackle[a,b,*]

[a]*Department of Microbiology and Immunology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29464, USA*
[b]*Department of Craniofacial Biology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29464, USA*

Received 3 April 2009; received in revised form 26 May 2009; accepted 30 May 2009

Abstract

Apoptotic cells are potent complement activators; and proposed mechanisms include IgM-mediated classical pathway activation, C-reactive protein (CRP)-mediated classical pathway activation, and IgM-mediated lectin pathway activation. While complement activation is beneficial in clearing apoptotic cells, the resulting complement-mediated inflammation may extend damage to the surrounding cells and tissues, as observed in ischemia/reperfusion injury. We previously engineered and characterized a single-chain Fv against C1q globular heads ($scFv_{QuVHVL}$) that blocked C1q binding to immobilized IgG and to IgG-sensitized cells, and thereby inhibited IgG-mediated classical pathway activation [Hwang H.Y., Duvall M.R., Tomlinson S., Boackle R.J., 2008. Highly specific inhibition of C1q globular-head binding to human IgG: a novel approach to control and regulate the classical complement pathway using an engineered single-chain antibody variable fragment. Molecular Immunology 45, 2570–2580].

In the present study, this $scFv_{QuVHVL}$ was examined for its ability to restrict complement deposition on apoptotic cells in the presence of fresh normal human serum (NHS). Interestingly, the addition of $scFv_{QuVHVL}$ to NHS decreased C1-mediated C4b deposition on apoptotic cells by 60% as compared to appropriate buffer-treated control serum. By inhibiting initiation of the early complement components, the subsequent C3b and membrane attack complex depositions were inhibited by 70%.

Apoptotic cells may acquire serum CRP, a known classical complement pathway activator. It was observed that $scFv_{QuVHVL}$ blocked C1 binding to CRP and blocked CRP-mediated classical pathway activation using an ELISA format. However, under the experimental conditions used, the addition of exogenous CRP to apoptotic cells did not further increase the levels of C4b, C3b, or MAC deposition significantly, suggesting predominance by other activation mechanisms, such as antibody-C1-mediated complement activation.

In summary, the results indicated that C1-mediated classical pathway activation was a highly significant mechanism for complement activation by apoptotic cells. In the future, specific inhibition of classical complement pathway

*Corresponding author at: Medical University of South Carolina, Room 442 Basic Science Building, 171 Ashley Avenue, Charleston, SC 29425, USA. Tel.: +1 843 792 2551; fax: +1 843 792 6433.
E-mail address: boacklrj@musc.edu (R.J. Boackle).

0171-2985/$ - see front matter
doi:10.1016/j.imbio.2009.05.010

activation by a humanized form of $scFv_{QuVHVL}$ may be useful in reducing inadvertent damage to healthy bystander tissue in a variety of acute, complement-mediated inflammatory conditions, including ischemia/reperfusion injury.

Keywords: Complement; Classical complement pathway; Apoptosis; Human C-reactive protein (CRP); Single-chain Fv; Inflammatory disease; Ischemia reperfusion injuries

Introduction

The complement system is instrumental in clearing immune complexes and damaged host tissues; however, excessive or poorly controlled complement activation may result in inordinate levels of inflammation and undesirable extensions of tissue damage. Thus, it is credible that therapeutics should be developed that selectively, specifically and temporarily inhibit individual complement components to control and prevent undesirable acute complement-mediated damage. Complement deposition is amplified at each sequential step of the cascade; therefore inhibition at the initiation of the classical complement pathway (i.e., at C1) is a logical tactic for preventing the extension of complement-mediated bystander tissue damage induced by C1 activation.

The first component of the cascade, the C1 macromolecular complex, is composed of one C1q molecule, two C1r ($C1r_2$) and two C1s ($C1s_2$) molecules (Boackle et al. 1979; Cooper 1985; Kishore and Reid 1999; Reid and Porter 1976; Schumaker et al. 1986; Sellar et al. 1992). The C1q subcomponent is a 460 kDa glycoprotein with an umbrella-like structure consisting of 6 A–B chains and 6 C–C chains (Yonemasu and Stroud 1972). Each chain consists of a short N-terminal region, followed by a collagen-like region and finally each chain becomes part of one of six C1q-terminal globular heads responsible for binding to exposed segments on the Fc region of antigen-bound IgM or IgG antibodies (Boackle et al. 1979; Gaboriaud et al. 2003; Kishore and Reid 1999; Kishore et al. 2003).

The best understood function of the C1q subcomponent is its integral non-covalent participation as the recognition unit within the macromolecular $C1qr_2s_2$ complex in the initiation of the classical complement pathway. Normally, the classical complement pathway begins when several of the globular heads of C1q engage a sufficient number of Fc regions of antibodies bound to antigenic surfaces, leading to C1q conformational changes. These conformational changes induce $C1r_2$ self-activation, which in turn activate the proximate $C1s_2$. While C1q is best known for binding to IgM and IgG, the classical pathway may also be activated when C1q binds to other ligands. One well-studied molecule that becomes involved in pathological situations is deposited C-reactive protein (CRP), an acute phase protein that binds to C-polysaccharides on microorganisms such as Pneumococcus, as well as phosphocholine exposed on damaged or apoptotic cells, and to modified low density lipoprotein (Agrawal 2005).

C1 activation is beneficial in the clearance of immune complexes and damaged cells, but it is also implicated in extending the pathogenesis of atherosclerosis (Saad et al. 2006; Singh et al. 2008), Alzheimer disease (Bradt et al. 1998; Sarvari et al. 2003; Selkoe and Schenk 2003), Type 2 diabetes (Zhang et al. 2007), and ischemia/reperfusion injuries. (Barrett et al. 2002; Griselli et al. 1999). Ischemia/reperfusion injury differs from the other conditions in that its effect on complement deposition is acute rather than chronic. Ischemic injury results in both necrosis and apoptosis (Krijnen et al. 2002; Rodriguez et al. 2002) and the injured tissue activates complement, thus amplifying proximate damage to normal tissues (Barrett et al. 2002; Griselli et al. 1999). Proposed mechanisms include classical complement pathway activation via CRP (Nijmeijer et al. 2001), IgM-mediated classical complement pathway activation (Zhang et al. 2004) IgM-mediated lectin complement pathway activation (Hart et al. 2005; Walsh et al. 2005; Zhang et al. 2006), and direct C1 activation via cardiolipin (Boackle et al. 1993; Peitsch et al. 1988; Rossen et al. 1994).

We recently reported that a single-chain antibody variable fragment against C1q globular heads, $scFv_{QuVHVL}$, was capable of inhibiting IgG-mediated complement activation (Hwang et al. 2008). Here we report that inhibition of C1 in normal human serum with $scFv_{QuVHVL}$ significantly decreased complement deposition on apoptotic cells, indicating a major role for C1 and the classical complement pathway in apoptotic cell clearance. Since both antibodies and CRP are implicated in classical pathway activation by apoptotic cells, and CRP and antibodies bind to different regions of C1q, we also investigated the ability of $scFv_{QuVHVL}$ to inhibit CRP-mediated classical complement pathway activation. It was observed that $scFv_{QuVHVL}$ blocked C1q binding to immobilized CRP and inhibited CRP-mediated C4b deposition.