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CD55 in rat male reproductive tissue: Differential expression in testis and expression of a unique truncated isoform on spermatozoa☆

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Abstract

CD55 is a key regulator of complement activation, expressed on most tissues and cells in man and other mammals. In the rat, alternative splicing in the gene encoding CD55 yields GPI-anchored (GPI-CD55) and transmembrane (TM-CD55) forms. Published Northern blot analysis indicated that while GPI-CD55 was broadly expressed, TM-CD55 was primarily expressed in the testis, although the precise site of expression was not identified. To clarify the distribution of CD55 isoforms in rat reproductive tissues, we first performed immunohistochemistry and Western blot analysis with an anti-rat CD55 mAb that recognized all reported CD55 isoforms, and a polyclonal immunoglobulin specific for TM-CD55. CD55 was absent in testis prior to puberty. Post-puberty, CD55 was expressed at high levels on all spermiogenic cells from step 6 spermatid onward, and on mature spermatozoa focussed on the acrosome, but was absent from support cells and early progenitors. Enzymatic digestion revealed that GPI-CD55 was predominant in testis and spermatozoa. Staining for TM-CD55 with specific immunoglobulin confirmed its absence from mature sperm and expression on spermatids only between steps 11 and 14 of development. GPI-CD55 on spermatozoa was of lower molecular weight than that in testis and other tissues; sequencing from spermatozoal mRNA identified a unique isoform of GPI-CD55 missing short consensus repeat 4. The predominant acrosome expression and presence of a unique, truncated isoform of CD55 on spermatozoa provides further support for the hypothesis that the acrosome is a highly specialized region in which closely regulated complement activation may contribute to reproductive function.

Keywords: Complement; Rodent; Reproductive immunology

1. Introduction

The primary role of complement (C) activation is to defend the body from microorganisms. However, uncontrolled C activation can be extremely harmful for the host. Membrane C regulatory proteins (CReg) exist to protect the host from the injury that can be caused by excessive C activation (Asch et al., 1986; Medof et al., 1987; Liszewski et al., 1991; Oglesby et al., 1992; Nose et al., 1990). Because of this important role, there are multiple CReg and each is broadly expressed in various tissues to provide efficient, cooperative regulation of C activation. Recently, other roles of CReg have been identified in addition to the established functions in C regulation (Harris and Morgan, 2004). For example, CD55 (decay accelerating factor; DAF), a key regulator of C activation acting at the C3 convertase level, has been shown to be a ligand for an activation-induced antigen (CD97) on leukocytes in human and mouse (Hamann et al., 1996; Qian et al., 1999) and a receptor for enteroviruses (Williams et al., 2004) and *E. coli* (Hudault et al., 2004) in human.

In male reproductive tissues in human and rodents, the distribution patterns of CReg are restricted compared with most other tissues (Simpson and Holmes, 1994; Harris et al., 2006; Mizuno et al., 2006). The C3 convertase regulator CD46 is expressed as a unique hypoglycosylated isoform on the acrosome in human sperm (Riley et al., 2002a), as a truncated protein lacking the first short consensus repeat (SCR) in New World monkeys (Riley et al., 2002b), and is absent from all tissues except testis in rodents (Tsujimura et al., 1998; Hosokawa et

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al., 1996; Mead et al., 1999). We recently showed that distribution of CD46 in rat testis was tightly restricted to spermatogenic cells and spermatozoa and to the developing acrosome in these cell types (Mizuno et al., 2004, 2005). It has been suggested that the altered isoforms and restricted expression of CD46 in rodents and primates serves to protect from pathogens that utilize CD46 as receptor, and retention on the acrosome is cited as evidence for an evolutionarily conserved role in fertilization (Riley et al., 2002a; Tsujimura et al., 1998; Hosokawa et al., 1996; Mizuno et al., 2004; Taylor et al., 1994). In man, CD55 is expressed as a GPI-anchored protein, broadly distributed in various tissues (Lublin and Atkinson, 1989). In the human male reproductive system, CD55 is found on late spermatogenic cells and on mature spermatozoa (Simpson and Holmes, 1994). The precise distribution of CD55 on human spermatozoa remains controversial, all agreeing that it is most strongly expressed in the acrosome, with some claiming weak but global expression on the cell membrane, while others find no expression on the membrane (Simpson and Holmes, 1994; Taylor and Johnson, 1996; Cervoni et al., 1993). In rodents, CD55 exists as multiple isoforms, either arising from distinct genes, or by alternative splicing of a single gene. In mice, the *cd55* gene is duplicated; the *cd55.1* gene product is broadly distributed, while expression of the *cd55.2* gene is restricted to testis and splenic dendritic cells (Lin et al., 2001). Each of the murine *cd55* genes can undergo alternative splicing to produce GPI-anchored, transmembrane and putative soluble forms of CD55 protein (Harris et al., 1999; Lin et al., 2001). In rats, a single *cd55* gene is spliced to give GPI-anchored (GPI-CD55), transmembrane (TM-CD55) and putative soluble forms (Hinchliffe et al., 1998; Miwa et al., 2000). Northern blot analyses have indicated that GPI-CD55 is the most abundant isoform in all tissues while the transmembrane form is significantly expressed only in the testis, although the precise site of expression is unidentified (Miwa et al., 2000). The unique expression of TM-CD55 in rat testis provoked the suggestion that TM-CD55 is selectively expressed on spermatozoa to provide a more stable protection for these cells, compensating for their limited capacity to synthesize proteins *de novo* (Miwa et al., 2001).

We recently reported that rat epididymal spermatozoa expressed CD55 strongly in the acrosome but also, more weakly, on the cell membrane (Mizuno et al., 2006). Crry was absent from spermatozoa and CD46 was acrosome restricted, leaving CD55 as the sole C3 convertase regulator available to protect the membrane in rat spermatozoa. It was therefore important to ascertain the precise expression profile of CD55 in developing and mature spermatozoa and to discover which of the isoforms are expressed in these cell types. Here we show that TM-CD55 is expressed transiently during spermatozoal development and is absent from mature spermatozoa. Spermatozoal GPI-CD55 is a lower molecular weight protein compared to testis and other tissues due to alternative splicing that deletes the fourth short consensus repeat (SCR). The data add to the growing body of evidence that C activation and regulation on the spermatozoal acrosome differ in many respects from other tissues, creating a unique environment where regulated and targeted C activation may contribute to reproductive function.

2. Materials and methods

2.1. Animals

Adults male and female Wistar rats (12–16 weeks), infant male Wistar rats (10–40 days) and adult male PVG and Lewis rats were humanely killed using UK Home Office approved methods and the tissues and blood were harvested for the following experiments.

2.2. Antibodies and reagents

The well characterized mAb against rat CD55, RDIII7, was generated by immunizing mice with a recombinant form of rat CD55 comprising the four short consensus repeat (SCR) domains (Spiller et al., 1999). Polyclonal (pc) anti-rat CD55 was generated by immunizing a rabbit with the same recombinant protein. Polyclonal antiserum specific for TM-CD55 was generated by immunization of rabbits with the peptide "CSNRSSDLQGKKKRENV" derived from the transmembrane specific C-terminal domain of TM-CD55 (Miwa et al., 2000) The peptide was synthesized and supplied by Eurogentec (Seraing, Belgium) and coupled to keyhole limpet haemocyanin (KLH) before immunization. Specific antibody was purified by affinity chromatography using the peptide immobilised on CNBr-activated Sepharose^{TM} 4B (Amersham Biosciences, Uppsala, Sweden). The mAb against rat CD46, MM.1, has been described previously (Mizuno et al., 2004). The mAb was biotinylated according to a published method (Guesdon et al., 1979). Polyclonal anti-rat Crry was generated in-house following established procedures and characterized as described (Mizuno et al., 2006).

FITC-labelled donkey anti-mouse IgG, rhodamine-labelled donkey anti-mouse IgG, rhodamine-labelled donkey anti-rabbit IgG, HRPO-labelled donkey anti-mouse IgG and HRPO-labelled donkey anti-rabbit IgG, were all minimal cross-reactivity against rat IgG and were purchased from Jackson ImmunoResearch Labs (Stratech Immunoresearch, UK). FITC-labelled goat anti-rabbit IgG was purchased from Sigma–Aldrich (Dorset, UK). An isotype-matched mAb (mouse IgG1), un-reactive against rat tissues, was made in house and used as a control for immunohistochemistry and other studies.

2.3. Preparation of spermatozoa

Motile epididymal spermatozoa were obtained by a modification of the "swim-up" technique for human spermatozoa, as described previously (Mizuno et al., 2004, 2005). Briefly, two cauda epididymi from an adult male Wistar rat were roughly minced in 2 ml of HAM F12 medium and gently shaken for 15 min at room temperature to release cells. The supernatant, without large cellular aggregates, was removed to a fresh tube and carefully overlaid with 2 ml of HAM F12 medium (Gibco-BRL, Paisley, UK). The tube was incubated for 60 min at 37 °C and the top 1.8 ml of HAM F12, which contained motile spermatozoa, carefully removed. "Swim-up" isolated spermatozoa were used for immunofluorescence (IF) analyses, for preparing