Cutting Edge: Inhibiting Measles Virus Infection but Promoting Reproduction: An Explanation for Splicing and Tissue-Specific Expression of CD46

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Membrane cofactor protein (MCP; CD46) regulates the complement cascade by inhibiting C3b and C4b deposited on self tissue. This function resides in the complement control protein repeats (CCPs), with CCPs 2–4 essential for regulation. MCP is expressed on the inner acrosomal membrane of human sperm, and Abs to CCP1 inhibit sperm-egg interactions. In somatic tissues, New World monkeys express an alternatively spliced form of MCP lacking CCP1. Although retaining complement-regulatory activity, this form is postulated to render these species less susceptible to strains of the measles virus whose hemagglutinin requires CCP1 and CCP2 for attachment. Using PCR, sequencing, Western blotting, and immunohistochemistry, we characterized MCP expression in the testes and sperm of two New World monkeys. In these species, sperm express MCP bearing CCP1. The germ cell-specific expression pattern of this domain strongly suggests an evolutionarily conserved role for MCP in fertilization. The Journal of Immunology, 2002, 169: 5405–5409.

Membrane cofactor protein (MCP; CD46) is a cofactor for the factor-I-mediated cleavage of C3b and C4b deposited on self tissue (1, 2). Human sperm express MCP on the inner acrosomal membrane (IAM) as a hypoglycosylated form that is not observed in other tissues (3–5). Sperm-specific abnormalities in MCP have been associated with infertility in humans (6, 7). Furthermore, Abs to MCP inhibit both binding and penetration of human sperm to zona-free hamster eggs (4, 8, 9) and to human zona (10). In particular, Abs to the first complement control protein repeat (CCP) of MCP inhibited binding of human sperm more efficiently than Abs to CCPs 2–4, which block complement regulatory activity (Fig. 1) (9).

MCP is a receptor for the measles virus and several other pathogens (2, 11–14). During an investigation of natural variations in the MCP gene that affect measles virus infection, Hsu et al. (15) examined eight species of New World monkeys covering seven of the 16 genera and all major families. They noted that erythrocytes of these New World monkeys did not hemagglutinate in the presence of the Edmonston strain of the measles virus and determined that this was due to the absence of CCP1, which, with CCP2, comprises the measles virus binding site in MCP (15–19). The lack of CCP1 is due to an alternative splicing event that was postulated to render the New World monkeys less susceptible to certain strains of the measles virus yet maintain MCP’s complement regulatory activity (13, 15, 20). We hypothesized that expression of MCP bearing CCP1 would be evolutionarily conserved on sperm of New World monkeys if MCP plays an important role in fertilization.

Materials and Methods

Tissue and sperm collection

Marmoset (Callithrix jacchus) samples were obtained from the Wisconsin Regional Primate Center (Madison, WI). Experimental protocols involving these animals were reviewed and approved by the Graduate School Animal Care and Use Committee of the University of Wisconsin, Madison, and were in accordance with National Institutes of Health and U.S. Department of Agriculture animal care guidelines. The Wisconsin Regional Primate Center is accredited by American Association of Laboratory Animal Care as part of the University of Wisconsin, Madison, Graduate School. Bolivian squirrel monkey (Saimiri boliviensis boliviensis) samples were obtained from the Squirrel Monkey Breeding and Research Resource at the University of South Alabama (Mobile, AL). For all experiments, samples from at least two different mature monkeys were examined. In each case, the marmoset and the squirrel monkey ejaculate and whole blood samples were collected from the same animal. For immunohistochemistry, sperm were allowed to swim up from fresh ejaculate and immediately subjected to the staining protocol. Human sperm samples were obtained from healthy donors and prepared as previously reported (3).

Abs, RNA and protein purification, Western blotting, and glycosidase treatment

The anti-MCP (CD46) rabbit polyclonal Ab was obtained from Millennium Pharmaceuticals (Cambridge, MA) courtesy of G. Yeh. Protein fractions and RNA were purified simultaneously from tissue samples or cell lines using the TRIzol reagent (Life Technologies, Grand Island, NY) (3). Western blots and glycosidase treatments were performed as previously described (3).

RT-PCR and cloning and sequencing of MCP transcripts

RT-PCR was performed as previously described (3). The sequence for the 5’ primer in the signal peptide was CCTCCCAGCCGGCCGAGTGT.
Acrosome reaction and immunohistochemistry

The acrosome reaction was performed as described (3). Freshly washed sperm, either acrosome-reacted (AR) or nonreacted, were stained in the fluid phase. The sperm were incubated with the primary Ab (rabbit anti-MCP polyclonal antiserum at 1/500 dilution in PBS/1% BSA) for 30 min at room temperature. The samples were pelleted at 1000 × g for 7 min and washed with PBS. The sperm were then incubated with a tetramethylrhodamine isothiocyanate-conjugated goat anti-rabbit IgG Ab (diluted 1/50 in PBS/1% BSA) for 30 min. The stained sperm were washed and resuspended in PBS.

Results and Discussion

MCP expressed by marmoset and squirrel monkey sperm migrates similarly to MCP from human sperm

Although MCP expressed in most human tissues forms a two-band pattern with Mr values between 51 and 68 kDa on SDS-PAGE, human sperm display a single band that has a faster Mr (48 kDa) (Fig. 2, lane 1) (21). This human sperm-specific form contains all four CCPs, the C exon of the serine, threonine, and proline-rich (STP) region and CYT-2 (the C2 isoform) (Fig. III) (3, 21). Its N-linked sugars are trimmed during spermatogenesis to less complex structures, accounting for the smaller molecular mass. MCP from blood cells, liver, and lung of the marmoset (Fig. 2, lanes 3–5) and the blood cells of the squirrel monkey (lane 8) is a single band migrating at 60 kDa. Hsu et al. (15) determined by Ab specificity that this protein lacks CCP1. Its STP and cytoplasmic tail regions (Fig. 1) were not evaluated. Other protein species were not observed by ourselves (Fig. 2) or Hsu et al. (13, 15).

MCP expressed by sperm of the marmoset and the squirrel monkey is a single band migrating at 48 kDa, similar to Mr to human sperm MCP (Fig. 2, lanes 1, 2, and 7). If MCP expressed on monkey sperm lacked CCP1, one would expect an ~10 kDa difference in molecular mass compared with the human protein. Therefore, the similar Mr, was an initial observation suggesting that CCP1 might be retained in MCP of the New World monkey sperm. The Western blot of MCP from the testes of the marmoset exhibits a doublet, with one band migrating at 60 kDa and the other at 54 kDa (Fig. 2, lane 6, arrows). Because the 60-kDa protein migrates identically to that observed on other cell types of the marmoset, it is likely derived from nongerm cells in the testes. Based on these data, we reasoned that the lower band at 54 kDa is derived from germ cells and contains CCP1. Presumably, this protein would undergo the same N-linked glycan “trimming” (3) to generate the hypoglycosylated form of MCP found on sperm (Fig. III, and Fig. 2, lane 2).

N-Linked glycosylation of MCP is similar for marmosets, squirrel monkeys, and humans

To further examine the monkey MCP, we digested protein lysates from these tissues with peptide N-glycosidase (PNGase)F, an enzyme that removes N-linked sugars. Upon digestion, the Mr of the human sperm protein decreases to 41 kDa (Fig. 3, lanes 1 and 2). This same decrease is observed for the sperm from both the marmoset and the squirrel monkey (Fig. 3, lanes 3–4 and 9–10). This

FIGURE 1. Diagram of MCP structure. The genomic organization is shown in Fig. 4C. There are three exons in the STP region whose translated products are referred to as A, B, and C. The C terminus of MCP consists of one of two possible cytoplasmic tails referred to as CYT-1 or CYT-2. MCP is expressed as isoforms that arise via alternative splicing of the STP and cytoplasmic tail regions (28, 29). I, Common human isoforms. In humans, the C region is constitutively expressed while the B region is alternatively spliced. Combining the BC or C isoform with either CTY-1 or CTY-2 gives rise to the four typical isoforms (BC1, C1, BC2, and C2) observed in human tissue. The A STP region is rarely observed in humans (22). II, Somatic monkey form. MCP from somatic tissues of New World monkeys lacks CCP1 (15) and, as will be shown, expresses the ABC STP region. III, Sperm-specific form. Spermatozoa of both humans (3) and New World monkeys, as will be shown, express the C2 isoform of MCP with trimmed N-linked sugars (3). CR, Complement regulation. MVB, Measles virus-binding site.

FIGURE 2. MCP protein expression patterns in humans and monkeys. Total protein extract (~10 µg) was separated by SDS-PAGE under nonreducing conditions, transferred, and then incubated with a rabbit anti-serum to MCP (1/5000 dilution). An HRP-linked donkey anti-rabbit Ab (1/3000 dilution) was used as a secondary Ab. The arrows indicate the doublet observed in the marmoset testes sample.

FIGURE 3. Treatment of MCP from selected tissues with PNGase. Protein samples (~15 µg) were incubated with PNGase for 24 h at 37°C (comparable results with a 1-h digestion). Samples were then Western blotted as described in Fig. 2. +, PNGase-treated samples.
result suggests that the structure of MCP on sperm is similar for both monkeys and humans, implying the presence of CCP1 in monkey sperm MCP.

Following PNGase digestion, the upper band of the marmoset testes sample aligns with the blood-derived protein, while the lower band aligns with the sperm band at 41 kDa (Fig. 3, lanes 7–8). The upper band protein correlates with the protein expressed on blood cells, while the lower band corresponds to the protein derived from germ cells (3). The faster \( M_t \) of MCP on the monkey sperm likely occurs secondary to a “trimming” of the \( N \)-linked sugars as the sperm mature and exit the testes (3). This trimming event has now been described for four other sperm proteins including decay-accelerating factor (DAF), a complement regulator closely related to MCP (reviewed in Ref. 3). We previously speculated that this processing of \( N \)-linked glycans might be essential to an egg–sperm interaction (3), and this idea is further supported by the apparent conservation of this process in the marmoset and squirrel monkey.

**CCP1 is present in mRNA from the testes of the marmoset**

Using RNA from marmoset tissues, we next performed RT-PCR and sequencing to determine whether the mRNA species were consistent with our interpretation of the protein data. Three primer sets were used (Fig. 4). The first set annealed in CCPs 2 and 4 (Fig. 4a). As expected, these primers produced a single band for all three tissues analyzed, and in each case, the sequence was that of CCPs 2–4. The second primer set annealed in the signal peptide and CCP4. It was designed to produce different bands, depending on the presence or absence of CCP1. In the testes, three distinct bands of 680, 580, and 500 bp were obtained (Fig. 4a). The sequence of the 500-bp mRNA species, also observed in the liver and lung, represents the MCP isoform lacking CCP1 (as was predicted from the band size). The 680-bp species is the anticipated size of the isoform containing CCP1, and this was confirmed by sequencing. Therefore, CCP1 of MCP is specifically retained in testicular mRNA of the marmoset.

The third band at 580 bp was not expected and sequencing established it as a splice variant possessing CCP1 but lacking the C-terminal half of CCP2 (exon 4) (Fig. 4c). CCP2 is the only CCP of MCP encoded by two exons. It would migrate \( \sim 5 \) kDa faster than the protein containing the whole CCP, and no protein correlating with this isoform has been observed. It is unlikely that this isoform would fold correctly, as it is lacking two of the cysteines required for the formation of the disulfide bonds. However, it is interesting to note that exon 4 codes for a part of the measles virus-binding site (15–19). This isoform may represent a less efficient evolutionary attempt at preventing measles virus infection, because the loss of exon 4 would also diminish complement regulatory activity (20).

The third primer set anneals to CCP4 and to CYT-2 and was designed to assess which STP and cytoplasmic tail region was expressed (Fig. 4b). Two bands from the testes were obtained, and the sequencing indicated that the lower band represents the C2 isoform (Figs. 1 and 4). The latter is the same isoform observed on human sperm. This result combined with those at the protein level establish that the C2 isoform is also the sperm-specific species of MCP in these New World monkeys.

The upper band produced by these primers is the ABC2 isoform. The major isoform expressed in the liver and the lung is also the ABC2 isoform, establishing that MCP bearing all three STP regions is expressed on tissues of the marmoset (Fig. 1I). This result accounts for why the \( M_t \) of monkey MCP in somatic tissues was 60 kDa, despite lacking CCP1, and is consistent with the \( M_t \) values obtained following digestion with PNGase F (Fig. 3). There is a low-level expression of BC and C isoforms in these tissues, based on mobility and sequencing of the minor bands in Fig. 4b. Whereas

**FIGURE 4.** Comparison of MCP mRNA splice variants in marmoset tissues. Using primers located in CCP2 and CCP4 (a), the signal peptide and CCP4 (a), or CCP4 and CYT-2 (b), the mRNA transcripts that code the isoforms of MCP can be distinguished. RNA samples from the indicated tissues were subjected to RT-PCR, and the products were separated in a 3% agarose gel. c, Genomic organization of the MCP gene and location of primers used in a and b. The gene consists of 14 exons and differential splicing of the STP and CYT exons gives rise to the major protein isoforms (Fig. 1). CCP2 is encoded by two exons (28, 29).
MCP bearing the ABC STP region has been observed in human tissues, it is rare, with most tissues expressing the BC and C STP regions (Fig. 1I) (22). In contrast, in these monkeys, it is the major isoform expressed.

MCP is expressed on the IAM in the marmoset
Human MCP is expressed on the IAM of sperm (Fig. 5). This membrane is exposed only after sperm have bound to the zona pellucida of the egg and undergone an exocytosis event referred to as the acrosome reaction. Only AR human sperm stain for MCP (Fig. 6e). The same pattern was observed for the marmoset sperm (Fig. 6g), indicating expression on the IAM.

A unique role for MCP in fertilization has been suspected for several reasons. First, because sperm express MCP solely on the IAM, it cannot protect these germ cells from complement in the female reproductive tract until after the acrosome reaction. However, sperm express two other complement regulators, DAF and CD59. CD59 expression is limited to the plasma membrane, and DAF is expressed on both the plasma membrane and the IAM. (23) Second, the hypoglycosylated form of MCP expressed on human and New World monkey sperm (Fig. 1II) is not observed in other tissues and is independent of the inherited polymorphism controlling the splicing of the STP exons. (3, 21). Third, in mice (24), rats (25), and guinea pigs (26), MCP is prominently expressed only in the testes. In these species, a different complement regulator known as Cry is widely expressed and has complement regulatory activity similar to that of MCP. This further suggests a pivotal role for MCP on sperm that cannot be fulfilled by simply replacing its complement regulatory activity (27). Fourth, sperm-specific abnormalities in MCP have been associated with infertility in humans (6, 7). Fifth, Abs to MCP, specifically those to CCPI, inhibit sperm-egg interactions (4, 8–10). These data, combined with those presented in this report, indicate that MCP likely plays a more direct role in fertilization. They are consistent with MCP being both a complement regulator and a participant in germ cell interactions. Moreover, these functions map to different regions of MCP, with CCPI likely important for reproduction and CCPs 2–4 necessary for complement regulation. Thus, an evolutionarily conserved role for MCP in reproduction is further implied by the male germ cell-specific retention of CCPI in New World monkeys.

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References

FIGURE 6. Localization of human and marmoset MCP on sperm. Acrosome reaction was induced with the calcium ionophore A23187. The rabbit polyclonal Ab to human MCP was used at a 1/500 dilution and the secondary Ab was a tetramethylrhodamine isothiocyanate-linked sheep anti-rabbit Ab at a 1/50 dilution. Secondary Ab controls were negative in all cases. a–d, Non-acrosome-reacted (NAR) sperm; e–h, AR sperm. a, c, e, and g, Fluorescent microscopy; b, d, f, and h, bright-field microscopy.


