Title
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Permalink
https://escholarship.org/uc/item/487498cq

Journal
Biology of Reproduction, 94(6)

ISSN
0006-3363

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Publication Date
2016-06-01

DOI
10.1095/biolreprod.115.137810

Peer reviewed
Sialylation Facilitates the Maturation of Mammalian Sperm and Affects Its Survival in Female Uterus

Xue Ma, Qian Pan, Ying Feng, Biswa P. Choudhury, Qianhong Ma, Pascal Gagneux and Fang Ma

ABSTRACT
Establishment of adequate levels of sialylation is crucial for sperm survival and function after insemination; however, the mechanism for the addition of the sperm sialome has not been identified. Here, we report evidence for several different mechanisms that contribute to the establishment of the mature sperm sialome. Directly quantifying the source of the nucleotide sugar CMP-beta-N-acetylneuraminic acid in epididymal fluid indicates that transsialylation occurs in the upper epididymis. Western blots for the low-molecular-mass sialoglycoprotein (around 20–50 kDa) in C57BL/6 mice epididymal fluid reflect that additional sialome could be obtained by glycosylphosphatidylinositol-anchored sialoglycopeptide incorporation during epididymal transit in the caput of the epididymis. Additionally, we found that in Cmah (CMP-N-acetylneuraminic acid hydroxylase)−/− transgenic mice, epididymal sperm obtained sialized-CD52 from seminal vesicle fluid (SVF). Finally, we used Gfp (green fluorescent protein)/+ mouse sperm to test the role of sialylation on sperm for protection from female leukocyte attack. There is very low phagocytosis of the epididymal sperm when compared to that of sperm coincubated with SVF. Treating sperm with Arthrobacter ureafaciens sialidase (AUS) increased phagocytosis even further. Our results highlight the different mechanisms of increasing sialylation, which lead to the formation of the mature sperm sialome, as well as reveal the sialome’s function in sperm survival within the female genital tract.

phagocytosis, sialic acid, sperm

INTRODUCTION
All cell surfaces in nature are covered by a glyocalyx, a dense “sugar coat” formed by a complex array of the oligo- and polysaccharides attached to glycoproteins and glycolipids [1]. Sperm are coated with a thick (~70 nm) glyocalyx rich in sialic acids (Sias) [2]. Sias are nine-carbon-backbone amino sugars abundantly found on mammalian cell surfaces and secretions [3]. These acidic sugars are important in a large number of biological functions ranging from either structure or function roles based on their negative charge to involvement in molecular recognition during development, immune regulation, and carcinogenesis [4]. There are over 50 different forms of Sias in nature, but the two most common Sias found in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Humans only express Neu5Ac as an ancient inactivating mutation of the Cmah gene and have abolished their ability to modify CMP-Neu5Ac into CMP-Neu5Gc [5]. The common linkage types of Sias to the underlying glycan chains are Sia α2-3 and Sia α2-6 [6]. A third linkage type, α2-8, is found, e.g., in cells of the central nervous system and sperm [7–9].

As with other mammalian cells, Sias are found as the outermost monosaccharide, capping the majority of glycans at the sperm cell surface. The sperm sialome refers to the total Sias present in and on sperm. We have previously quantified Sias on mature sperm cells and found that each sperm bears tens of millions of Sia molecules [10]. The sperm sialome is established during spermatogenesis, during epididymal maturation, and by incorporation of sialylated seminal fluid components into the sperm membrane during ejaculation [11, 12]. As allogeneic cells, sperm face important challenges once introduced into the female reproductive tract [13]. In fact, female immune factors both humoral and cellular lead to the rapid demise of the vast majority of sperm. The leukocytic
reaction unleashes large numbers of leukocytes in the uterus shortly upon copulation [14, 15]. Antibodies and complements are also components of female reproductive tract secretions [16], and highly sialylated epididymal glycoproteins such as beta defensins have been shown to mask sperm antigens to antibody binding [17]. The complement system also relies in part on appropriate sialylation of cells to detect self and inhibit activation based on factor H binding [18], and as such, appropriate sialylation could be as important for sperm survival as the presence of multiple-complement down-regulating molecules such as CD55 and CD59 [19]. Insufficient decoration with Sias is likely to put sperm at increased risk of female immune intolerance.

Sia content of mammalian sperm has been shown to correlate positively with protection from phagocytosis but negatively with the capacity of sperm to bind to zona pellucida of the ovum [20, 21]. It appears that Sia decoration of sperm is subject to temporal modulation during sperm maturation. Existing studies of Sia modulation in sperm have mostly relied on indirect measures of Sia gain, including transferase activity and ganglioside content [12, 22]. We here profiled the sialome of sperm from spermatogenesis in the testis, in transition through the epididymal lumen and maturation, and finally in contact with seminal fluid during ejaculation. Also, we investigated the mechanisms responsible for the addition of the sialome during sperm maturation, and revisited the role of sperm sialylation for protection after insemination.

MATERIALS AND METHODS

Animal Care and Use

The research was conducted using C57BL/6 mice, Cmah/−/− mice, and Gfp (green fluorescent protein)/+/+ mice following the approval of the University of California (UC) San Diego Animal Care and Use Committee. Animals used in these studies were maintained under UC San Diego IACUC Protocol #S01227 and euthanized according to the principles and procedures described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sperm Collection and Preparation of Sperm Extracts

Sperm were harvested from the cauda epididymis of euthanized 12- to 20-wk-old males. Cauda epididymis was crushed and kept on a shaker at room temperature (RT) for 10 min, then centrifuged for 30 sec at 500 × g. Sperm were washed with Biggers-Whitten-Whittingham medium (BWW; pH 7.5: 91.06 mM NaCl, 4.78 mM KCl, 1.71 mM CaCl, 1.71 mM KH₂PO₄, 2.44 mM MgSO₄, 7H₂O, 5.55 mM glucose, 0.25 mM sodium pyruvate, 21 mM HEPES at pH 7.4, 25 mM NaHCO₃, 21.5 mM sodium lactate) by centrifugation at 800 g for 1 min and subjected to a swim-up procedure in 500 μl of physiological BWW and placed in a humidity chamber at 37°C until used for assays.

Fluids with sperm from different epididymal regions were collected. The fluids with sperm from different epididymal regions were collected. The samples were gently washed three times with BWW. The supernatant was pooled, concentrated, and then centrifuged to remove the pellet. Protein was quantified and kept at −20°C until used for assays.

In Vitro Seminal Vesicle Fluid Incubation with Sperm

Seminal vesicles were isolated from blood vessels and accessory glands by careful dissection. The gland fluid contents were manually expressed into microcentrifuge tubes. Twenty microliters of seminal fluid was then mixed with 100 μl of physiological BWW and placed in a humidity chamber at 37°C for 10 min. The suspension was centrifuged at 1000 × g for 5 min and the supernatant was collected for sperm incubation; 10⁶ sperm were added to 50 μl diluted seminal vesicle fluid (SVF) and incubated at 37°C and 5% CO₂ for 15 min [25].

Retrieving Leukocytes of Female Mice 15 h after Mating and Coincubation with Sperm

A single male (C57BL/6) was caged with two females in estrus stage overnight. The following morning the females were examined for the presence of vaginal plugs as an indication of successful mating. Females were euthanized 15 h after mating, the whole uterus was excised, the two horns were separated, and each horn was clamped shut with forceps. The uterine horns were flushed with 200 μl PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄, 1.8 mM KH₂PO₄) using a 28-gauge syringe. Live sperm were removed by swim-up method to isolate leukocytes. The leukocytes were then coincubated with the Gfp+/− sperm group at 37°C and 5% CO₂ for 4 h.

Western Blot

Mouse membrane sperm proteins were extracted following the protocol of Shetty et al. [24]. Mouse SVF proteins were separated by loading SVF directly on 10% SDS-PAGE (Bio Rad). Proteins were either transferred to PVDF membrane (Bio Rad) or stained with Coomassie stain (Bio Rad). For detection of α2-3 and α2-6 sialylglycoprotein, the membranes were blocked with 1% bovine serum albumin (BSA) in Tris-buffered saline with Tween (TBST; 0.1%) and incubated overnight at 4°C. After washing, the blots were incubated for 30 min with 1:6000 biotinylated Sambucus nigra lectin (SNA; Vector Laboratories) and Maucaia amurensis lectin 2 (MAL-II; Vector Laboratories) in 1% BSA TBST, washed, then incubated with horseradish peroxidase (HRP)- conjugated streptavidin (Vector Laboratories) at concentration of 1:100 000 for 1 h at RT. Meanwhile, mild NaIO₄ treatment was used as control (membrane were washed with H₂O and incubated with 20 mM NaIO₄ in PBS [pH 6.5] at RT 30 min in the dark). For Neu5Gc staining, blots were incubated for 1 h at RT in 1:5000 affinity purified chicken IgY (Sialix, Inc.) in TBST, 0.5% fish gelatin (Sigma-Aldrich), and 2 μg/ml of purified BSA (double blocking). Blots were washed and incubated with secondary HRP-conjugated donkey anti- chicken antibody (1:5000; Biologend) for 1 h at RT. Blots were developed using Pierce luminescent substrate (Thermo Scientific).

Analysis of Flow Cytometry and Immunofluorescence

To assess lectin binding, sperm were fixed with 3% freshly thawed paraformaldehyde (PFA) for 20 min at RT, washed with PBS, and then blocked with 1% BSA TBST. Biotinylated lectins of SNA and MAL-II were used at a concentration of 1:1000 at RT for 30 min and detected with streptavidin conjugated with Alexa Fluor 555/488 secondary antibodies (Biologend) at a concentration of 1:1000 at RT for 30 min. To evaluate CD52 expression, spermatozoa were fixed with freshly thawed 3% PFA for 20 min at RT, washed with PBS, and then blocked with 1% BSA PBS. Anti-mouse CD52 antibody (Biologend) was used at 1:400 at 4°C for 1 h, and secondary donkey anti-mouse antibody conjugated with Alexa Fluor 647 (Biologend) was used at 1:500 at 4°C for 1 h. For APC/CY7-F4/80, the mixed cells after coincubation were washed with PBS, then incubated with the antibody at 1:500. Stained sperm were analyzed by flow cytometry on a BD FACSCalibur. A smear of stained sperm was counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1:500000; Sigma-Aldrich), and also made for microscopy and microphotography on an applied precision Deltavision inverted deconvolution system (Zeiss).

Immunohistochemistry

The male genital tissues including testis and epididymis were harvested, then frozen by surrounding with Optimum Cutting Temperature compound (Vector Lab) and immersed in a bath of dry ice and 2 methyl butanol slush. Five-micrometer sections were prepared by sectioning on a cryostat microtome. Sections were fixed with acetone for 20 min, washed with PBS, and then blocked with 1% BSA PBS. Sections were incubated with 1:500 diluted of Neu5Gc antibody (Sialix, Inc.) were used at a concentration of 1:500 for 1 h at RT. Meanwhile, mild NaIO₄ treatment was used as control (membrane were washed with H₂O and incubated with 20 mM NaIO₄ in PBS [pH 6.5] at RT 30 min in the dark). For Neu5Gc staining, blots were incubated for 1 h at RT in 1:5000 affinity purified chicken IgY (Sialix, Inc.) in TBST, 0.5% fish gelatin (Sigma-Aldrich), and 2 μg/ml of purified BSA (double blocking). Blots were washed and incubated with secondary HRP-conjugated donkey anti- chicken antibody (1:5000; Biologend) for 1 h at RT. Blots were developed using Pierce luminescent substrate (Thermo Scientific).

Determination of CMP-Sia by High-Performance Anion-Exchange Chromatography-Pulsed Amperometric Detector

The regional epididymal fluids were detected with high-performance anion-exchange chromatography-pulsed amperometric detector (HPAEC-PAD; Dionex ICS 3000 system). Elutions were carried out at 1 ml/min with the
Arthrobacter ureafaciens Sialidase Treatment

Arthrobacter ureafaciens sialidase (AUS; EY Laboratories) was used at 15 mU/100,000 sperm cells for partial desialylation. The treatment time was 20 min.

Statistical Analysis

Data sets were analyzed for statistical significance using a one-way ANOVA test. All values shown are means ± SEM. P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001 were considered statistically significant versus control.

RESULTS

Sialome Profile of Mouse Sperm at Different Stages of Development

There is some information about the sperm sialome in some mammalian species [26]. To understand the sialome on sperm during the maturation process, we determined the sialome of mouse sperm using lectin and immunohistochemical methods. We stained mouse testes and epididymides using SNA (specific for Sia \(^{2-6}\)), MAL-II (specific for Sia \(^{2-3}\)), and anti-Neu5Gc antibody (polyclonal IgY raised in chicken against Neu5Gc). We found no Sias expressed on testicular sperm during early developmental stages except for Sia \(^{2-3}\), which was strongly expressed on spermatoocytes. This indicates that the sialome first appears on spermatocytes as Sia \(^{2-3}\) of Neu5Ac. Mature mouse sperm expressed more than 20% of their Sias as Neu5Gc. The histochemistry in Figure 1B indicates that most of the Neu5Gc appears on sperm only after it leaves the testes. Once sperm were outside the testes, there was an incremental increase of Sias on sperm from caput to caudal region of the epididymis, including \(^{2-3}\) and \(^{2-6}\) Sias (Fig. 1, A and B). There was also abundant Sia on the adjacent epithelia of the epididymal duct (Fig. 1B), from which certain sialoglycoconjugates might be acquired by the membrane of maturing sperm. In order to explore the acquisition of the sperm sialome, we used fluorescent lectin staining followed by quantification using flow cytometry. Analysis of sialylation levels of epididymal sperm from proximal to distal regions (caput to cauda) provided evidence for an incremental increase in Sia coating of the mouse sperm surface (Fig. 1C).

Neu5Ac Is Transferred to Sperm from Luminal CMP-Neu5Ac During Epididymal Transit

It has been previously reported that there are different concentrations of sialyltransferase in the lumen of rat epididymis from caput and cauda [27]. The coincubation of exogenous CMP-Neu5Ac and sialyltransferase with mouse sperm showed that Sia can be added to the sperm surface in vitro from exogenous CMP-Neu5Ac [12]. These findings are provocative, as CMP-Neu5Ac is not usually found outside of cells. To identify the presence of CMP-Neu5Ac in epididymal fluid, which is an absolute prerequisite for extracellular sialyltransferase function, we collected epididymal fluid from different regions of the epididymis (Fig. 2A) separated into caput 1, caput 2, corpus, and cauda. We performed determination and quantification of extracellular CMP-Sia by HPAEC-PAD using a standard CMP-Neu5Ac positive control. A blank control and female mouse uterine fluid were set as negative and positive control respectively. A small amount of CMP-Neu5Ac, but no CMP-Neu5Gc, was detected in wild-type (WT) mice. The concentration was highest in caput 2 and corpus compared with testis, caput 1, and cauda (Fig. 2, B and C), suggesting that CMP-Sia is transferred to sperm by sialyltransferase mainly in epididymal caput and corpus during transition.

Glycosylphosphatidylinositol-Anchored Sialoglycoproteins Are Obtained by Sperm During Epididymal Transition

Known mechanisms for the gain of sialoglycoconjugates by cells include the incorporation of sialylated glycosylphosphatidylinositol (GPI)-anchored glycopeptides or glycoproteins and the incorporation of gangliosides (sialylated glycosphingolipids) [22]. A further possibility is the recently demonstrated coating of maturing sperm by a beta defensin (Bin1b in rat, Defb22 in mouse, and DEFB126 in primates) with a typical cationic domain and a tail domain that contains numerous sialylated O-glycans [28]. The configuration of the mature sperm sialome thus likely reflects epididymal sialylation as well as incorporation of GPI-anchored sialoglycopeptides, gangliosides, and sialylated defensins from epididymal fluid. To test the degree of acquisition of GPI-anchored sialoglycopeptides during epididymal transit, we isolated epididymal fluid and used lectin binding and high-affinity anti-Neu5Gc antibody staining of sialoglycoprotein separated by reduced SDS-PAGE. Because this procedure is very sensitive and less specific, we used Neu5Ac \(^{2-3/6}\)-human sera albumin (HSA) as positive control. We found sialoglycoproteins throughout the epididymis, but the amount and composition varied between fluids from different segments of the epididymis (Fig. 3A). Glycoproteins with \(^{2-3}\) Sia were mainly expressed in caput 2 as low molecular mass (around 20–50 kDa), whereas glycoproteins with \(^{2-6}\) Sia were mainly expressed in caput 1 as low molecular mass (around 20–50 kDa). Staining of Western blots for the Sia Neu5Gc in WT mice epididymal fluid revealed abundant presence of glycoproteins decorated with this Sia throughout (Fig. 3, B–D). Thus, the expression of low-molecular-mass sialoglycoprotein indicates that additional sialome could be obtained by GPI-anchored sialoglycopeptide incorporation during epididymal transit, and it appears more likely that this transfer occurs in the caput rather than in the corpus and cauda of epididymis.

Sialome Gain from SVF During In Vitro Incubation

Prior to their release by ejaculation, sperm are stored under physiological stimulation in the epididymis for the final maturation processes. The contact with seminal plasma provides an additional opportunity for the modulation of the sperm sialome, as sperm can assimilate gangliosides and GPI-anchored sialoglycolipids from seminal fluid [22]. The quantification of gangliosides requires large amounts of glycolipid, making it difficult to measure the total gangliosides that sperm acquire from seminal fluid. To investigate the mechanism of the addition of GPI-anchored sialoglycoprotein, we focused on the GPI-anchored sialoglycopeptide CD52, a highly sialylated glycopeptide with sperm-specific glycoforms, which has been demonstrated to be obtained by mouse sperm only after ejaculation [29]. In contrast, CD52 is expressed in the epididymis in humans. We took advantage of the Cmah−/− transgenic mouse, which lacks the Sia Neu5Gc and has only Neu5Ac [5]. We performed in vitro incubation of epididymal sperm and SVF following the reported procedure [30] but used sperm from Cmah−/− mice coincubated with SVF from WT...
mice, which contains CD52 decorated with Neu5Gc. We measured the change in sialylation by staining the sperm with anti-Neu5Gc and anti-CD52 antibodies on Western blot and by flow cytometry. Neu5Gc detected on the Cmah−/− sperm that were coincubated with WT mice SVF would reflect Sia from SVF.

We found striking differences in staining of Western blots for a glycoprotein of ∼20 kDa between epididymal sperm, sperm after coincubation with SVF, and SVF alone (Fig. 4A). We found that CD52 from SVF was added to epididymal sperm, and that Sia α2-3 and Neu5Gc were acquired at the same time (Fig. 4A).

The double staining with anti-CD52 and anti-Neu5Gc antibodies confirmed the addition of sialylated CD52 (Fig. 4B). Lectin staining indicates that more α2-6 linked Sia was obtained with CD52 (Fig. 4C). Neu5Gc was clearly acquired by Cmah−/− sperm after incubation with WT SVF, but these sperm exhibited an overall reduced sialome compared to WT sperm (Fig. 4, D and E). The Neu5Gc signal is predominantly localized to the sperm head; this finding suggests that sialylation acquired by GPI-anchored CD52 and ganglioside during ejaculation mostly affects that region of the sperm. It appears that final Sia addition to the ejaculated sperm disproportionally involves the sperm head.

**Sperm Sialylation Protects Sperm from Female Macrophage Attack**

The mechanism by which a mature sialome protects sperm from female immune response in the uterus remains unknown. The millionfold reduction in sperm numbers in the uterus is mostly due to the action of female leukocytes. Although the mechanism of phagocytosis of sperm by uterine leukocytes remains unclear, here we chose to study direct recognition and phagocytosis by macrophages. We retrieved leucocytes from female mice 15 h after mating, when large numbers of leukocytes have infiltrated the uterus [31], and used sperm of Gfp+/+ males (Fig. 5A), which have green fluorescent midpieces, as a tracer for monitoring phagocytosis by macrophages. Additionally, we used AUS treatment to cleave
most of the Sias from the sperm membrane and tested the effect of such a loss of Sias. Paradoxically, there is very low phagocytosis of the epididymal sperm when compared with that of sperm coincubated with SVF, which had a 46% phagocytosis rate (Fig. 5, B and C). If Sias were removed by AUS treatment from sperm coincubated with SVF, phagocytosis increased even further (57%; Fig. 5, B and C). Factors from SVF thus both increase the likelihood of sperm being phagocytosed by uterine macrophages and mitigate that risk by contributing sialoglycoproteins.

DISCUSSION

As the most common terminal monosaccharides on mammalian cells, Sias form the “molecular frontier of the glyocalyx.” This predisposes this family of sugars for playing...
likely a good indication of the magnitude of adversity faced by sperm. Surviving the onslaught of female immune factors is an absolute prerequisite for sperm to reach the upper oviduct for fertilization. The down-regulation of major histocompatibility complex (MHC) class I molecules on mature sperm allows them to avoid histocompatibility-based immune responses by female MHC I-restricted cytotoxic T cells. Abundant sialylation likely facilitates tolerance by female innate pattern-recognition molecules, both secreted molecules such as complements (e.g., factor H, which binds to Sia and prevents complement deposition) [32] and cell-bound molecules on female leukocytes (e.g., Sia-recognizing immunoglobulin superfamily lectins [Siglecs], which bind to Sia and can inhibit immune cell activation) [33]. In addition, decoration with terminal Sia may play a role in masking potential antigenic sperm molecules until after sperm have undergone capacitation and sperm have approached the egg and its vestment. Such masking has been suggested for estrogen and N-acetyl-5-methoxytryptamine receptors on the sperm midpiece, which contribute to the regulation of sperm motility by inhibiting the breakdown of ATP [34, 35]. Similarly, unmasked tyrosine phosphorylated chaperone proteins overlaying the acrosome of capacitated sperm might mediate sperm zona binding [36]. Masking of potent sperm antigens such as ph20 by highly sialylated DEFB126 has also been demonstrated [28].

Changes in sperm sialylation level en route to fertilization have been reported for several decades (Table 1); however, the mechanisms involved remain unknown. At the beginning of spermatogenesis, spermatogonia, or immature germ cells, divide mitotically to produce diploid cells termed spermatocytes. Subsequently, after meiotic cell division, spermatids are released from the testes into the epididymis, where they undergo a process of maturation and ultimately acquire motility. Sperm surfaces are further modified by absorption of coating molecules from seminal fluid during ejaculation. Regarding the gain of Sia during sperm development, a recent study reported Sia decoration on sperm during spermatogenesis in the testes [37], and earlier studies have reported sperm acquire Sia during epididymal transit and maturation, a process that involves sialylation by luminal sialyltransferase particularly in the caput epididymis [8, 9, 38]. Additional increases in sperm sialylation were shown to occur via incorporation of secreted GPI-anchored sialoglycoproteins, especially CD52, and also incorporation of gangliosides (Sia containing glycosphingolipids) from seminal fluid during ejaculation. We found that there was free CMP-Neu5Ac (the donor sugar nucleotide used by sialyltransferases to add Sias to glycoproteins) from seminal fluid during ejaculation. It was reported that in female golden hamsters, the highest levels of leucocytes (average 1 497 000) was found 12–14 h after mating with a fertile male, mainly because of the presence of semen itself and the large numbers of spermatozoa in the uterus. The rapid disappearance of spermatozoa from the uterus on the first day following mating is ascribed to the phagocytotic action of leucocytes and drainage through the cervix, and the mechanism of how leucocytes recognize the sperm remains unclear. On the other hand, it is important to maintain an adequate number of incapacitated sperm that can move into the oviduct to meet the ovum. Therefore, protecting multiple roles in cellular recognition processes. As foreign cells, mammalian sperm face numerous challenges during their transit through the female reproductive tract en route to fertilization [13]. That insemination with hundreds of millions of individual sperm is required to fertilize one or a few ova is likely a good indication of the magnitude of adversity faced by sperm. Surviving the onslaught of female immune factors is an
FIG. 4. Sialylation of epididymal sperm by exposure to SVF. A) Samples: 1, epididymal sperm; 2, epididymal sperm coincubated with SVF; 3, SVF. Left panel: sperm sialoglycoproteins in the samples. Proteins around 20 kDa molecular mass were presented on SDS-PAGE gel by Coomassie staining (loading: 10 µg protein/lane). Middle panel: presence of CD52 in the samples. Samples were blotted with anti-CD52 antibody (loading: 10 µg/lane). Right panel: detection of Sia α2-3 and Neu5Gc linked with CD52 in the samples. Proteins were detected with specific lectin binding and anti-Neu5Gc antibody (loading: for Sia α2-3, 10 µg/lane; for Neu5Gc, 2 µg/lane). B) Expression of Sia α2-3/6 and Neu5Gc on CD52 in sperm samples before (green dots) and after (red dots) coincubation with SVF.
FIG. 5. Evidence for the role of sialylation and its protection from phagocytosis by macrophages. A) Distribution of green fluorescence on Gfp+/+ mouse sperm. The sperm of transgenic Gfp+/+ mouse were directly observed under fluorescent microscope. The green fluorescence was mainly concentrated in the middle section of the sperm. B) Detection of phagocytosed Gfp+/+ sperm by fluorescence-activated cell sorting (FACS). (Gfp positive control: Gfp+/+ sperm; F4/80 positive control: macrophage). AUS treatment of sperm: 15 U/100,000 sperm cells; 10,000 cells were counted for each group. The quantized phagocytosis data are presented in each frame. Low phagocytosis of the epididymal sperm was observed comparing with that of the sperm coincubated with SVF. C) Quantification of phagocytosis of sperm by macrophage according to the FACS data. The data are expressed as mean ± SEM (n = 5; **p < 0.01; ***p < 0.001).

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TABLE 1. Evidence for modulation of sperm Sia content.

after (red dots) coincubation with WT SVF. The samples were analyzed by fluorescence-activated cell sorting (FACS) using double staining (Negative control signals: gray dots). Fifty thousand sperm cells were counted for each group. C) Quantification of the different sialylation linkage (Sia α2-3/6) on CD52 with fluorescent intensity based on the FACS data. The data are expressed as mean ± SEM (n = 5). D) Evidence of analyzed sialylation on Cmah+/+ mouse epididymal sperm coincubated with WT SVF detected by anti-Neu5Gc antibody by FACS. Fifty thousand sperm cells were counted for each group. E) Image of analyzed sialylation on Cmah-/− mouse epididymal sperm coincubated with WT SVF detected by anti-Neu5Gc antibody (green), DAPI (blue) counterstained. Bars = 5 µm and 2 µm (inset).
sperm from excessive phagocytosis by leucocytes is crucial. In our study (Fig. 5), it was identified that epididymal sperm without complete sialylation were not recognized by uterine macrophages. It was reported that the 54-kDa sialoglycoprotein with the aid of Sia residues was implicated in the protection of sperm from phagocytosis [20]. However, epididymal sperm that were desialylated by treating with AUS, which partly cleaves the Sia from sialoglycoprotein and ganglioside, underwent phagocytosis at a much higher rate than sperm incubated with SVF without AUS treatment. This indicates that intact sialylation plays a key role for the recognition of sperm by macrophages or neutrophils. That the desialylation of sperm increases phagocytosis by macrophages suggests sialylation might mask some molecules to protect the sperm from being chased by leucocytes.

In our study, we profiled sialylation addition during sperm maturation and provide evidence that the sialome may contribute to the protection of sperm from female uterine immune responses. On the other side, our reported study found that sialidases are present on the surface of mouse and human sperm, which are activated during capacitation [39]. The coexpression of sialyltransferase and sialidase on sperm might give some clues to explore how and why Sias change in the lifespan of sperm, similarly to the “yin and yang” mechanism, to keep the balance of sialylation and desialylation. Future studies should focus on further characterizing the mechanism of sialylation and its contribution to sperm function and immune evasion.

ACKNOWLEDGMENT

We gratefully acknowledge Ajit Varki for use of Cmah−/− mice, and Liwen Deng for the English polishing of this manuscript.

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