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Further Characterization of Reproductive Abnormalities in mCd59b Knockout Mice: A Potential New Function of mCd59 in Male Reproduction

Xuebin Qin,† Martin Dobarro,‡ Sylvia J. Bedford,§ Sean Ferris,† Patricia V. Miranda,‡ Wenping Song,† Roderick T. Bronson,§ Pablo E. Visconti,‡ and Jose A. Halperin³*†

CD59 is a GPI-linked membrane protein that inhibits formation of the membrane attack complex of complement. We reported recently that mice have two CD59 genes (termed mCd59a and mCd59b), and that the targeted deletion of mCd59b (mCd59b−/−) results in spontaneous hemolytic anemia and progressive loss of male fertility. Further studies of the reproductive abnormalities in mCd59b−/− mice reported in this study revealed the presence of abnormal multinucleated cells and increased apoptotic cells within the walls of the seminiferous tubules, and a decrease in the number, motility, and viability of sperm associated with a significant increase in abnormal sperm morphologies. Both the capacitation-associated tyrosine phosphorylation and the ionophore-induced acrosome reaction as well as lutetizing hormone, follicle-stimulating hormone, and testosterone serum levels were similar in mCd59b−/− and mCd59b+/+. Surprisingly, the functional deficiency of the complement protein C3 did not rescue the abnormal reproductive phenotype of mCd59b−/−, although it was efficient in rescuing their hemolytic anemia. These results indicate that the male reproductive abnormalities in mCd59b−/− are complement-independent, and that mCd59 may have a novel function in spermatogenesis that is most likely unrelated to its function as an inhibitor of membrane attack complex formation. The Journal of Immunology, 2005, 175: 6294–6302.

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4 Abbreviations used in this paper: MAC, membrane attack complex; LH, lutetizing hormone; FSH, follicle-stimulating hormone; Crry, complement-related receptor protein; DAF, decay accelerating factor; MCP, membrane cofactor protein.
which these Abs produced decidual focal necrosis with C3 de-
position and neutrophil infiltration in pregnant mice (10). 

In the male reproductive system, spermatogenesis begins at pu-
berty, long after the immune system has developed the ability
to distinguish self from “nonself”, and sperm-surface Ags can trigger
an immune/complement response that would compromise the po-
tential of the sperms for successful fertilization. Thus, it is not
 surprising that, in addition to a protective blood-testis barrier, a
variety of nonspecific and specific complement inhibitors, includ-
ing CD59, are present at high concentrations in the seminal fluid,
and that sperm cells express multiple complement regulators that
prevent complement activation and functional MAC formation
(3–6, 11, 12). In the seminal plasma, CD59 is present in a GPI-
anchored, prostasome-associated form that can “deliver” CD59
molecules to the sperm cell (13), and is also highly expressed on
sperm plasma membranes (6). Incubation of sperm with neutral-
izing anti-CD59 Abs and human serum results in sperm immobi-
 lization and lysis, whereas sperm with functional CD59 are pro-
tected (14).

Recently, we and others have demonstrated that mice have two
different CD59 genes (mCd59a and mCd59b) (15–17), whereas
most other rodents and humans studied have only one. mCd59b is
highly expressed in testes at the level of mRNA and protein (18),
a finding that suggested a possible testsis/reproduction-specific
function of mCd59b. To study the function of CD59 and comple-
ment in mouse models of human diseases, particularly the function
of mCd59b, we generated mCd59b−/− mice by homologous re-
combination. These mice express a hemolytic anemia phenotype
comparable to human paroxysmal nocturnal hemoglobinuria, and
are complement independent, and that mCd59 may have a novel
inhibitor of MAC formation.

**Materials and Methods**

**Mice**

The following animal studies have all been reviewed and approved by the
Harvard Medical School Institutional Animal Care and Use Committee
(Animal Welfare Assurance no. A3431-01; International Animal Care and
Use Committee approval date: January 10, 2005).

**mCd59b−/−.** Both mCd59b−/− and mCd59b+/- littermate mice used for
these experiments were offspring derived from intercrossing mCd59b−/− in
a B6/129 mixed genetic background, all characterized by PCR genotyping
(7). To avoid the influence of multiple matings on sperm analysis, the
experimental littersmates were not mated with females.

**mCd59b−/−C3−/−.** To obtain mCd59b−/−C3−/− (mCd59b and C3 dou-
ble knockout) and mCd59b−/−C3−/- (both mCd59b and C3 wild type)
littermates, we first crossed mCd59b−/− (in B6/129 mixed genetic
background) and C3−/− (in B6 genetic background; purchased from The Jack-
on Laboratory) mice to generate mCd59b−/−C3−/− (7, 19). Both
mCd59b−/−C3−/- and mCd59b−/−C3−/− mouse lines were used for experi-
ments to obtain pregnancy by intercrossing mCd59b−/−C3−/−, selected
by genotyping as described in Refs. 7 and 19.

**Collection of blood and sperm**

Blood was collected in tubes containing EDTA by cardiac puncture using
precooled needles and syringes to avoid complement activation. Testes and
epididymides were dissected, the cauda epididymis was separated, and
sperm were collected by squeezing one cauda epididymis from each mouse
into 1 ml of pretreated HEPES-buffered saline solution containing 1%
BSA (10 mM HEPES, 150 mM NaCl, 0.01 g/ml BSA (pH 7.4)). Epidid-
yal tissue was removed and the solution was filtered through a mesh filter
into a conical centrifuge tube.

**Serum levels of luteinizing hormone (LH), follicle-stimulating
hormone (FSH), and testosterone**

LH and FSH were determined by RIA at the National Institute of Diabetes
and Digestive and Kidney Disease on a fee-for-service basis as described
in Ref. 20. Testosterone was measured by ELISA following the manufac-
turer’s recommendations (BioCheck).

**Histological analysis of testes**

Testes were fixed in Bouin’s solution, 5-mm slices were embedded in
paraffin, and 6-μm sections were stained with H&E using routine proce-
dures (21). Quantitative analyses were performed on histological sections
obtained from fixed, sectioned testes using a Nikon Eclipse TE2000-E
microscope equipped with a Spot digital camera. The number of multinu-
cleated cells in seminiferous tubules was counted blindly by two indepen-
dent investigators from two sections of each testis at ×20 magnification.

**Immunohistochemical analysis**

**Detection of C3 and C9 deposition in testes.** Eight-micrometer serial sec-
tions from paraffin-embedded tissue samples were stained with: 1) goat
anti-mouse C3 Ab (ICN Pharmaceuticals) (22) or 2) rabbit anti-rat C9 Ab,
which cross-reacts with mouse C9 (kindly provided by Dr. P. Morgan,
University of Wales) (22). Either anti-goat for anti-C3 or goat anti-rabbit
for anti-C9 conjugated to peroxidase were used as secondary Abs follow-
ing established procedures (23). Two independent investigators blindly
counted the samples based on the intensity differences between stainings
by each specific Ab and nonimmune IgG.

**Detection of apoptotic cells in situ.** Apoptotic nuclei in tissue sections
were identified by a TUNEL technique using an in situ apoptosis detection
kit (Apoptag; Chemicon International) according to the manufacturer’s in-
structions (24, 25). The sections were counterstained with toluidine blue,
dehydrated with ethanol and xylene, and coverslipped. The number of
TUNEL-positive nuclei in 40 ×200 microscope fields per sample was re-
corded blindly by different investigators.

**Sperm analysis**

**Progressive motility.** The percentage of progressively motile sperm
was immediately evaluated by placing a 10-μl drop of the diluted sperm sus-
pension between a prewarmed glass slide and a coverslip, and visually
counting under a phase contrast microscope at ×200. Morphologically normal sperm and sperm count.

A total of 10 μl of the sperm suspension was diluted 1/10 into saline-buffered formaldehyde in a
microcentrifuge tube. The relative distribution of morphologically normal
and abnormal sperm was evaluated in a wet slide under a phase contrast
microscope by counting a total of 200 sperm per sample at ×1000. The sperm abnormalities recorded included detached heads, abnormal head
shapes, bent midpieces, droplets, coiled tails, other abnormalities, and ima-
mature sperm forms (round cells) (26, 27). We defined abnormal heads as
the sum of detached heads and abnormal sperm heads (type Ia-Ib-IIc-
IIIa-IIlb, IV, and V) (27).

A 10-μl drop of the above suspension was used to count sperm cells on
a hemocytometer as described in Ref. 28.

**Viability.** To differentiate live from dead sperm cells we used the live/dead
sperm viability kit (SYBR 14 + propidium iodide; Molecular Probes) follow-
ing the manufacturer’s recommendations. Briefly, 5 μl of a 10-fold
dMSO dilution of the SYBR 14 dye was mixed with the sperm suspension
and incubated for 5–10 min at 37°C in the dark. Then, 5 μl of the pro-
pidium iodide solution was added and incubated for an additional 5–10
min. A total of 200 cells were counted for viability evaluation in wet
samples under a fluorescence microscope where propidium-iodide stained
dead cells a fluorescent red.

**Sperm function analysis**

**Capacitation.** Sperm capacitation was evaluated by the protein tyrosine
phosphorylation assay (29, 30). Cauda epididymis were dissected and
placed in a petri dish containing bicarbonate-free Wittens medium to allow
sperm to swim out. Total sperm recovered per mouse was recorded. Sperm were capacitated for 1.5 h in Wittens medium supplemented with 20 mM NaHCO₃ and 5 mg/ml fatty-acid-free BSA (31). Control sperm were kept in medium without any supplement. Sperm extracts were obtained by boiling for 5 min with 1% SDS and subjected to SDS-PAGE followed by immunoblot with anti-phosphotyrosine mAb (4G10; Upstate).

Acrosome reaction. Acrosome reaction was induced by addition of 2 μM calcium ionophore (A23187) during the last 10 min of incubation. Acrosomal staining was performed using Alexa 488-conjugated peanut agglutinin (32) (Molecular Probes).

Measurement of C activity with hemolytic assay
An aliquot of mouse blood was analyzed on an Advia 120 autoanalyzer (Abbott Laboratories) as previously described in Ref. 7.

Assessment of the hematologic profile
An aliquot of mouse blood was analyzed on an Advia 120 autoanalyzer (Abbott Laboratories) as previously described in Ref. 7.

Statistical analysis
Mean difference of multinucleated cells, apoptotic cells, total sperm, and viable sperm, as well as motility, viability, and the percentage of sperm with abnormal heads between experimental groups were compared by Student’s t test.

We compared the median levels using the Wilcoxon rank sum test and we compared the proportions in the extremes of the distributions using Fisher’s exact test. Specifically, we compared proportions with hemoglobin levels <13 g/100 ml of blood (2 SD in mCd59b+/+ C3+/+) and proportions with reticulocyte counts greater than 4.3% (2 SD in mCd59b+/+ C3+/+).

Results
Testes histology, apoptotic cells, and complement deposition
Testis histological comparison between mCd59b+/+ and mCd59b−/− littermate mice at 6 and 10 mo of age revealed that 33% of mCd59b−/− mice had abnormal multinucleated cells within the walls of seminiferous tubules (mean number of abnormal multinucleated cells in mCd59b−/− = 10 ± 8 (n = 6 per age group)); these cells were absent in all mCd59b+/+ mice analyzed (n = 6 per age group) (Fig. 1). The TUNEL assay performed on testicular paraffin sections from 6-mo-old mCd59b−/− and mCd59b+/+ mice revealed the presence of a significantly greater number of apoptotic, TUNEL-positive germ cells in mCd59b−/− mice (Fig. 1, D, F, and G) than in their age-matched mCd59b+/+ control mice (Fig. 1, D and E). The comparison of mCd59b+/+ and mCd59b−/− littersmates of different ages (4, 6, and 10 mo old) showed that there was no difference in the testes to total body weight ratio, and no evidence of testicular atrophy or of inflammatory cells in the seminiferous tubules (data not shown). Also, immunostaining of testicular sections with either anti-C3 or

FIGURE 1. Presence of abnormal multinucleated cells and increased apoptotic cells in mCd59b−/− testis. A–C, Presence of abnormal multinucleated cells (red arrow) within the wall of the seminiferous tubules of mCd59b−/−. A, ×10 magnification; B and C, ×20 magnification. D–G, There are significantly more apoptotic cells in mCd59b−/− seminiferous tubules (D, F, and G) compared with mCd59b+/+ (E). The cells were blindly counted by two different investigators.
anti-C9 Abs did not show an increased complement deposition in mCd59b<sup>−/−</sup> testes (data not shown). Thus, the presence of abnormal multinucleated cells and of an increased number of apoptotic germ cells, the main histological findings in the testes of mCd59b<sup>−/−</sup>, do not seem to be associated with an increased complement/MAC deposition, as one would expect from the functional deletion of CD59.

**Sperm abnormalities in mCd59b<sup>−/−</sup>**

Comparative analysis of sperm at 3 mo of age showed that relative to mCd59b<sup>+/+</sup>, mCd59b<sup>−/−</sup> mice have a significant decrease in the total number of live sperm (Fig. 2A), and in the percentage of viable sperm (Fig. 2C). This decrease in critical sperm parameters in mCd59b<sup>−/−</sup> was associated with a significant increase in the percentage of abnormal sperm heads (Fig. 2C).

Sperm quality parameters progressively worsened; at 6 mo of age, both the number of total and live sperm (Fig. 2B), and the percentage of motile and viable sperm were all significantly decreased, whereas the percentage of abnormal sperm heads was significantly higher in mCd59b<sup>−/−</sup> mice (Fig. 2D). Fig. 2, E–G, show examples of abnormal sperm head morphologies frequently observed in mCd59b<sup>−/−</sup> mice. These results together with the abnormal histology findings indicate that the progressive loss of male fertility in mCd59b<sup>−/−</sup> mice may be due to abnormal spermatogenesis.

**Normal hormonal levels and capacitation-associated tyrosine phosphorylation in mCd59b<sup>−/−</sup>**

Fig. 3 shows that there were no significant differences in the serum levels of the critical reproduction-related hormones FSH (Fig. 3A) and LH (Fig. 3B); levels of testosterone were also similar in mCd59b<sup>−/−</sup> and mCd59b<sup>+/+</sup> males (data not shown). Thus, the progressive loss of male fertility and testicular abnormalities in mCd59b<sup>−/−</sup> mice do not seem to be caused by low levels of hormones that affect spermatogenesis.

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**FIGURE 2.** Comparison of the sperm parameters between mCd59b<sup>−/−</sup> and mCd59b<sup>+/+</sup> mice. A and B, Comparing total and live sperm of mCd59b<sup>−/−</sup> with that of mCd59b<sup>+/+</sup> at the age of 3 (A) and 6 mo (B). C and D, Comparison of the mobility, viability, and the percentage of abnormal sperm heads from mCd59b<sup>−/−</sup> with that of sperm from mCd59b<sup>+/+</sup> at the age of 3 (C) and 6 mo (D). *p < 0.05. Each group contained six males. ■, mCd59b<sup>−/−</sup>; □, mCd59b<sup>+/+</sup>. E–G, Increase in the number of abnormal heads of sperm in mCd59b<sup>−/−</sup> sperm morphologic abnormalities: detached head (arrow) and class III head (E), class IIIa head (F), and class IIIb head (G).
Both sperm capacitation and the acrosome reaction are physiological processes required for successful fertilization. Capacitation is associated with an increase in protein tyrosine phosphorylation. Indeed, Western blot analysis with specific Abs against phosphotyrosine has become an accepted method to assess capacitation (23–30). In other cell types, CD59 has been found to be physically connected to the membrane tyrosine phosphorylation machinery; if this were the case in sperm cells, CD59 could play a role in sperm capacitation (29, 30, 34). In other cell types, CD59 (C3) deficiency does not rescue the sperm abnormalities in mCd59b−/− mice.

C3 deficiency does not rescue the sperm abnormalities in mCd59b−/−

To investigate further whether the role of CD59 in spermatogenesis is dependent on an efficient complement system, we generated mCd59b+/+/C3+/+, mCd59b−/−/C3+/+, mCd59b+/+P C3−/−, and mCd59b−/−/C3−/− littersmates by intercrossing mCd59b−/−/C3+/+ followed by genotyping, as described in Materials and Methods. The functional deficiency of C3 in the different combinations involving the C3−/− genotype was confirmed by measuring the serum complement activity as described in (33). Sperm analysis at 6 mo of age revealed that in both mCd59b−/−/C3+/+ and mCd59b−/−/C3−/− there was a similar decrease in the total and viable sperm number, as well as in sperm motility and viability as compared with sperm from mCd59b−/−/C3+/+ (Fig. 4, A and B). In contrast, the functional deficiency of C3 rescued the hemolytic anemia phenotype that we originally described in mCd59b−/− mice (7), as shown in Table I. Moreover, 17% of mCd59b−/−/C3−/− mice had a hemoglobin value below 2 SD below the mean in wild-type mice (i.e., below 13 g/dl), a fraction significantly higher than that in the mCd59b−/−/C3−/− (4.5%) or in the mCd59b+/+P C3−/− (3.9%) groups (Fig. 5A). Similar results were obtained for reticulocyte counts: 15.8% of mCd59b−/−/C3+/+ littersmates had a reticulocyte count 2 SD above the mean of their mCd59b−/−/C3+/+ wild-type counterparts, a fraction that is also significantly higher than that in
FIGURE 4. The C3 deficiency does not rescue the sperm abnormalities in mCd59b−/−. A, Significant decrease in the number of total or live sperm from either mCd59b−/− C3+/+ or mCd59b−/− C3−/− compared with mCd59b+/+ C3−/− at 6 mo of age (n = 8). Live sperm number equals total number of sperm times the percentage of live sperm. B, Significant decrease in sperm motility or viability of either mCd59b−/− C3+/+ or mCd59b−/− C3−/− compared with mCd59b+/+ C3−/− at 6 mo of age (n = 8). * Comparing mCd59b−/− C3+/+ with mCd59b−/− C3−/− (p < 0.05). #, Comparing mCd59b−/− C3−/− with mCd59b+/+ C3−/− (p < 0.05).

Discussion

A critical role of complement in reproduction has been experimentally characterized by two recent studies showing the deleterious effect on the fetus of unrestricted or increased complement activation, as seen respectively in pregnant mice after either functional deletion of Crry (8, 9) or injection of anti-phospholipid Abs (10).

Table I. C3 deficiency rescues the hemolytic anemia in mCd59b−/−a

<table>
<thead>
<tr>
<th>Mouse Genotype</th>
<th>Hemoglobin (g/dl)</th>
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<tr>
<td>mCd59b+/+ C3+/+</td>
<td>14.95 ± 1.23</td>
</tr>
<tr>
<td>mCd59b−/− C3+/+</td>
<td>14.2 ± 2.21</td>
</tr>
<tr>
<td>mCd59b−/− C3−/−</td>
<td>14.82 ± 0.89</td>
</tr>
</tbody>
</table>

a Mice are from 3–6 mo old.

b Comparison between mCd59b+/+ C3−/− and mCd59b+/+ C3+/+, p < 0.01 (Student’s t test).

c Comparison between mCd59b−/− C3+/+ and mCd59b−/− C3−/−, NS, p > 0.1.

d Comparison between mCd59b−/− C3−/− and mCd59b+/+ C3−/−, p < 0.05.

e Comparison between mCd59b−/− C3+/+ and mCd59b+/+ C3−/−, p < 0.05.

FIGURE 5. C3 deficiency rescues the hemolytic anemia of mCd59b−/−. A, Seventeen percent of mCd59b−/− C3+/+ mice (n = 107) had 2 SD below the mean of their mCd59b+/+ C3−/− wild-type counterparts (<13 g/dl), a proportion significantly higher than the 4.5% found in mCd59b−/− C3−/− (n = 77) or the 3.9% found in mCd59b+/+ C3−/− littermates (n = 75). B, A total of 15.8% of mCd59b−/− C3−/− littermates had a reticulocyte count 2 SD above the mean of their mCd59b+/+ C3−/− wild-type counterparts (4.3%), a proportion significantly higher than the 4.5% present in mCd59b−/− C3−/− or the 3.9% detected in mCd59b+/+ C3−/−. * Fisher’s exact test: mCd59b−/− C3−/− with mCd59b+/+ C3−/− (p < 0.05); #, Fisher’s exact test: mCd59b−/− C3−/− with mCd59b+/+ C3−/− (p > 0.05). C, Complement activity in mCd59b−/− (B6/129 genetic background) was assessed by the fractional lysis of Ab-sensitized rabbit RBC mediated by each mouse serum (MS) assayed at different concentrations (10% and 20%). M1, M2, M3, M4, M5, M6, M7, and M8, individual mCd59b−/− mice; P, commercially available mouse serum; and HI, heat-inactivated mouse serum.

FIGURE 6. No significant changes in the sperm quality and quantity of C3-deficient mice. A, The number of viable sperm from mCd59b+/+ C3−/− is not significantly different from mCd59b+/+ C3−/−, but higher than that from mCd59b+/+ C3+/+ at 6 mo of age (n = 8). B, Neither motility nor viability from mCd59b+/+ C3−/− is significantly different from mCd59b+/+ C3−/−, but is higher than that from mCd59b+/+ C3−/− at 6 mo of age (n = 8). * p < 0.05.
A potential role of complement in sperm development, maturation, and fertilization (38) has long been suspected, but its formal demonstration has remained elusive. Because complement regulatory proteins, including CD59, decay accelerating factor (DAF), membrane cofactor protein (MCP), and S-protein, are highly expressed in spermatozoa and present in seminal plasma (5, 6, 39), it has been postulated that they could be critical in protecting sperm against complement attack in the male reproductive tract, and/or play physiological roles in spermatogenesis/spERM maturation. However, several molecularly engineered mice already developed to study the function of complement regulatory proteins, including two mDAF knockout mice (40), and mCd59a- (33) and S-protein-deficient mice (41) did not show any major fertility problem. Several in vitro studies suggested a role for MCP in sperm-egg interaction (42), but the targeted deletion of mouse MCP did not affect their reproductive efficacy (43). Together, these studies illustrate that in mice, DAF, mCd59a, MCP, and S-protein are not individually essential for fetal development/survival or for protecting sperm from complement activation in the male or the female reproductive system. In contrast, the targeted deletion of the mCd59b gene, resulting in the down-regulation of mCd59a and functional deficiency of mCd59b, leads to a progressive loss of male fertility, as we have reported in (7).

The results of the work presented in this study show that mCd59b−/− mice have a significantly lower number and compromised quality of sperm, associated with the presence of abnormal multinucleated cells, and an increased number of apoptotic cells within the seminiferous tubules. The multinucleated cells appear to be arrested spermatogonia that have divided without cytokinesis, an indication that spermatogenesis does not progress normally in vivo, as evidenced by the presence of multinucleated and apoptotic cells in the seminiferous tubules. These results are consistent with a defect in spermatogenesis and would explain the intriguing loss of male fertility that progressively occurs in mCd59b−/− mice. Interestingly, this spermatogenesis defect seems to result from a complement-independent function of C5b9 because 1) it is not associated with an increased deposition of MAC in the seminiferous tubules and 2) the functional deficiency of C3, which for all practical purposes makes mice complement insufficient, does not rescue the abnormal testicular phenotype, although it does rescue the complement-dependent hemolytic anemia (Figs. 4–6 and Table I).

Regarding the rescue of the hemolytic anemia of mCd59b−/− by C3 deficiency, it is worth noting that this phenotype does not have a 100% penetrance in vivo, although 100% of the animals tested exhibited a significantly increased sensitivity to complement-mediated lysis assayed against a pool of complement-efficient mouse serum (7). This lower penetrance of the phenotype in vivo is not unexpected because the hemolytic anemia in mCd59b−/− is the net result of the complement activity in the plasma of the individual mouse and the level of complement restriction due to the functional absence of Cds9. Assuming that all other complement regulatory factors are similar among individual mCd59b−/− mice, the main remaining variable that might influence the penetrance of the hemolytic anemia phenotype is the complement activity in each mouse. It has been established that there are significant differences in the levels of complement activity in different strains of mice, between males and females and also among same gender within the same genetic background (Fig. 5C) (33, 44, 45). The known difference in complement activity among individual mice is the most likely explanation for the observation that mCd59b−/−/C3++/− mice as a group exhibit anemia, i.e., a significantly lower blood hemoglobin value than normal littermate controls, but in only 17% of them, hemolysis is sufficient to bring this value below 2 SD of the mean in wild-type mice. In summary, C3 deficiency rescues the hemolytic anemia but not the abnormal reproductive phenotype of mCd59b−/− mice, indicating that the later is most likely complement independent.

At present, we cannot completely rule out the alternative possibility of a local production of the C3 complement component that could take place in the testes of C3 knockout mice via an alternative splicing product of C3. However, this alternative explanation seems unlikely because 1) MAC deposits were absent in the testes of mCd59b−/− mice; 2) a study designed to investigate the role of local complement production in renal transplantation using the same C3 knockout mice clearly indicates that there is no local production of C3 in the kidneys of these mice (46); and 3) the C3 knockout mice are also deficient in acylation-stimulation protein, a cleavage product of adipocyte C3 that contributes to fat tissue metabolism (47).

The successful reproduction of young mCd59b−/− males, and the demonstration of normal capacitation-associated tyrosine phosphorylation and ionophore-induced acrosome reaction in mCd59b−/− sperm, strongly support the interpretation that mCd59b may play a complement-independent role in spermatogenesis rather than in fertilization. This is consistent with the evidence that expression of mCd59b in spermatozoa progressively increases during their development and maturation (17, 18). Our in vivo data showing decreased motility of sperm in mCd59b−/− is consistent with the previous demonstration by two different groups that spermatozoa ejaculated from healthy human donors lost their motility after exposure to anti-human CD59 Abs (48, 49).

Although this study does not define the complement-independent role of CD59 in reproduction, it is tempting to speculate that CD59 might protect sperm and/or testicular germ cells from attack by a yet not characterized mechanism independent of complement. One recent example of an alternative function in reproduction for a well-known protein is the finding that angiotensin converting enzyme, a well-characterized zinc peptidase, plays an unexpected biological role in reproduction cleaving GPI-anchored proteins from the plasma membrane (50). Alternatively, the role of CD59 in reproduction could be related to the localization of CD59 in the sperm lipid rafts. Recently, Cross et al. (51) have shown that, in noncapacitated sperm, CD59 localizes to lipid rafts, and that it redistributes after disruption of lipid rafts during the capacitation process. The molecular mechanism of this postulated new role of CD59 in reproduction is currently under investigation in our laboratory.

The number of multinucleate and apoptotic cells was significantly increased in mCd59b−/− as compared with mCd59b+/+ mice (Fig. 1, A–D). The underlying mechanism of increased multinucleate and apoptotic cells in mCd59b−/− needs further investigation. Of note, the number of apoptotic cells was increased in some mCd59b−/− (four of six) but not all mCd59b−/− (Fig. 1D). This heterogeneous phenotype may be due to a different proportion of B6 and 129 genetic background in our experimental mice. It is well accepted that the genetic background largely influences phenotypic expression. To minimize the impact of difference genetic backgrounds, we used littermates (derived from crossing heterozygous pairs); however, this does not totally eliminate differences in genetic background that may affect phenotypic expression.

The progressive nature of the loss of male fertility in mCd59b−/− is intriguing. It indicates that mCd59 does not play a role that is indispensable for sperm development/survival, in which case one would expect the male infertility to be early and absolute from the beginning of the mouse male fertile cycle, rather than progressive. Even though we observed a significant reduction in the total number of normal, motile sperm at the early age of 5 mo in mCd59b−/− (Figs. 2 and 3C), the density of normally motile sperm at that age seems to be above the minimal threshold required
for a successful fertilization. In contrast, the density of normal sperm at the older age of 10 mo is not only further reduced (Fig. 5) but apparently below the fertilization threshold, explaining why older mice become completely infertile.

CD59 is a conserved gene universally expressed in most animal species (17). Mice seem to be unique in expressing two Cd59 genes, whereas all other species express only one (17). The presence of two Cd59 genes in mice appears to be the result of gene duplication rather than loss of one copy in the genome of humans and other species. This interpretation is based on our finding of early transposon or L1 retro-transposon repeat sequences located in the 5′ flanking region of both mCd59a and mCd59b genes in the mouse genome (17). These transposon repeat sequences are now considered as mobile or transportable genomic elements that have contributed to the evolution of species through different genomic pathways (52). Also, retro-transposition is believed to increase the probability of gene duplication and to generate insertional mutations responsible for some genetic diseases (53). Therefore, it seems possible that retro-transposons caused the gene duplication event that resulted in two Cd59 genes in the mouse genome (17). A combination of a complement-regulatory and a reproduction-specific function of mCd59b would have conferred a selective advantage that preserved the presence of both genes in the mouse genome. However, it is also possible that the reproduction-specific function is shared by both mCd59a and mCd59b mouse genes. The published work on mCd59a does not mention any reproduction abnormality (33, 44); this may be due to the absence of a reproduction-specific function in the mCd59a gene or to compensation by the mCd59b gene, which is highly expressed in testis. In mCd59b−/−, residual mCd59a might not compensate for the loss of a reproductive-specific function in testes because 1) mCd59a does not have the reproduction-specific function, 2) the level of expression of residual mCd59a in testis is not high enough to compensate, or 3) there is a down-regulation of mCd59a, as we have documented at the level of mRNA (see Fig. 5a in Ref. 7, and more recently by FACS analysis of mCd59b RBC, using mCd59a- and mCd59b-specific Abs (X. Qin et al., manuscript in preparation) (18). The issue of the reproduction-specific function of the mCd59 genes will be resolved by the availability of mCd59a and mCd59b double-knockout mice followed by knockin rescue experiments with either gene. Although mCd59b has a slightly higher homology with human CD59 than mCd59a (44 and 41%, respectively) (16), the available structural information does not allow us to ascertain whether mCd59a or mCd59b is the conserved gene in the human genome. At the functional level, it will be of great interest to determine whether CD59 also plays a physiological function in human sperm development.

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Disclosures

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References


