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Differential expression of complement regulatory proteins decay-accelerating factor (CD55), membrane cofactor protein (CD46) and CD59 during human spermatogenesis

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SUMMARY

We have examined the distribution of the complement (C) regulatory proteins CD59, membrane cofactor protein (MCP) and decay-accelerating factor (DAF) on mature sperm and compared expression of these proteins in parallel both during spermatogenesis and in the prostate. Enhanced immunoperoxidase staining and radioimmunoassay confirmed that C regulators are differentially expressed on sperm; CD59 was strongly expressed on the surface of acrosome intact sperm while MCP and DAF appear to be located primarily on the inner acrosomal membrane. While the MW of CD59 on sperm is typical of other systems, we confirm that in addition to a novel 40,000–46,000 MW MCP protein, sperm also express a novel 55,000 MW DAF product. Examination of normal testis by immunostaining revealed that although C regulators are differentially expressed within the germinal epithelium, all three proteins were present on the acrosomal region of condensing spermatids. We show that novel, low MW forms of MCP and DAF are expressed in normal testis membranes but are absent from testis membranes obtained from patients undergoing gender reassignment surgery in whom the germinal epithelium is diminished. Novel MW C3 convertase regulators are therefore associated with differentiating germinal epithelium. Typical CD59 components were also present on normal testis membranes confirming that CD59 is acquired during spermatogenesis. We demonstrate that the prostatic epithelium, in addition to MCP, expresses CD59 but not DAF. By comparison with CD59, therefore, our studies suggest that DAF may be acquired only in the testis. Overall, our data suggest that, on leaving the testis, sperm express the repertoire of C regulators required for protection from C during their transit through the male and female reproductive tracts.

INTRODUCTION

Three membrane-bound proteins decay-accelerating factor (DAF), membrane cofactor protein (MCP) and CD59 function to prevent autologous complement (C)-mediated damage initiated through activation of either the alternative or classical C pathways.[1,2] These regulators act at two distinct levels within the C pathway. DAF and MCP both act at the level of the C3 convertases; DAF acts reversibly preventing formation of C3 convertases and accelerating their decay[3] while MCP acts irreversibly as a cofactor for factor I-mediated cleavage of C3b and C4b.[4] CD59 interacts with the terminal C pathway components C8 and C9 directly regulating the formation of the cytolytic membrane attack complex (MAC).[5–7]

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Abbreviations: DAF, decay-accelerating factor; GPI, glycosylphosphatidylinositol; MAC, membrane attack complex; MCP, membrane cofactor protein; TBS, Tris-buffered saline.

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DAF, MCP and CD59 are known to be expressed at relatively high levels on the fetally derived placental epithelium suggesting that these proteins may play an important role in the maintenance of human pregnancy.[8] In addition, however, C regulators have recently been observed on human sperm suggesting a wider role for these proteins in reproductive success.[9–13] The female reproductive tract is known to contain functionally active C[14,15] and C regulatory proteins may therefore be important in protecting sperm from C-mediated damage in this potentially hostile environment.

Expression of MCP, the first of the C regulatory proteins to be reported on sperm, was found to be localized to the inner acrosomal membrane of the sperm head.[9] This functionally active MCP product differs from MCP proteins expressed on other tissues in that it is present on sperm in the form of a single 40,000 MW component. This is some 10,000–20,000 lower in MW than other characterized MCP species.[10] In contrast, sperm CD59 was found to be broadly distributed on both the head and tail of mature sperm and its MW was similar to that of CD59 expressed in other tissues.[11] The distribution of DAF on sperm is currently less clear. While Rooney *et al.*[11] found that DAF was broadly distributed on both the head and tail of sperm, in two

recent reports, Bozas *et al.*[12] and Cervoni *et al.*[13] found DAF only on the acrosomal region. However, although Bozas *et al.*[12] reported that the MW of DAF on sperm was typical of DAF in other tissues, Cervoni *et al.*[13] found that sperm DAF exhibited a relatively low MW.

Overall, studies to date suggest that C regulatory proteins may be differentially distributed on mature sperm but the origin of this differential expression during sperm maturation is at present undetermined. Expression of MCP in normal testis appears to be associated with the acrosomal region of condensing spermatids.[9] Cervoni *et al.*[16] have reported that the MW of testicular MCP corresponds to that of mature sperm and that it is encoded by an unusual MCP cDNA. However, the expression of MCP in the testis may be more complex as Seya *et al.*[17] have recently reported a 60,000 MW testicular MCP protein which is comparable in size with a soluble form of the regulator found in seminal plasma. The expression of the glycosyl-phosphatidylinositol (GPI)-anchored C regulators CD59 and DAF during normal sperm maturation has not been reported in the same detail. DAF is known to be expressed in the normal testis[13] but expression of CD59 at this site has not been described although, interestingly, it has recently been shown that sperm may acquire additional CD59 through interaction with extracellular organelles present in seminal plasma termed prostasomes.[18]

In this study we have further examined the expression of DAF, MCP and CD59 on mature sperm in order to clarify their relative distribution patterns. We have also examined expression of the three regulators in parallel both during spermatogenesis in the testis, and in the prostatic epithelium which is already known to contribute CD59 to seminal plasma. We show that, while all three regulators are expressed in testis, each of the proteins displays a distinct distribution on the differentiating germinal epithelium. Finally, in order to establish whether C regulators are associated with maturing sperm we also examine the expression of C regulators in testes from individuals where spermatogenesis is suppressed.

MATERIALS AND METHODS

Tissues

Adult human testes were obtained fresh at surgery from men undergoing biopsy to assess sperm production and from patients undergoing orchidectomy. These tissues were kindly provided by Mr C. Gingell and colleagues (Southmead Hospital, Bristol, U.K.). Prostate was obtained at post-mortem and as surgical specimens from men undergoing trans-urethral resection of the prostate. Testes were also obtained at surgery from three male trans-sexual patients undergoing penile resection for gender reassignment and we are grateful to Mr J. O. Dalrymple (Dept. of Psychiatry, Charing Cross Hospital, London, U.K.) for his kind co-operation in obtaining this material. High-dose oestrogen therapy, taken by these patients in their attempts to acquire female characteristics, results in suppression of spermatogenesis and atrophy of the germinal epithelium. Hormone therapy was ceased at least 6 weeks prior to surgery. Term placentae were obtained from apparently normal full-term deliveries.

For immunostaining, small blocks of tissue were placed in OCT embedding compound (Lab Tek Products, Napperville, IL), frozen in liquid nitrogen-cooled isopentane (2-methylbutane) on cork boards and stored in liquid nitrogen. For biochemical studies, tissues were either used fresh or were snap frozen and stored in liquid nitrogen.

Preparation of sperm

Human semen was obtained from normal fertile donors and liquified at 37° for 30 min. Motile sperm were recovered from semen by a swim-up procedure.[19] Briefly, entire ejaculates were layered under Biggers, Whitten and Whittingham medium (BWW)[20] in a 10-ml plastic centrifuge tube and incubated at 37° for 1 hr at an angle of about 45°. The upper 1·2 ml of the BWW layer was removed and sperm pelleted by centrifugation. For radioimmunoassay (RIA), sperm samples were resuspended in BWW, divided equally and incubated for 1 hr at 37° under 95% air:5% CO_2. During this incubation acrosome reaction was promoted in one-half of the sample by addition of the calcium ionophore A23187 (free acid, Boehringer plc, Lewes, U.K.) to a final concentration of 5 μM. These preparations were kindly provided by the staff of the Seminology Laboratory (Reproductive Medicine Unit, Dept. of Obstetrics, Bristol University, U.K.).

Immunohistology

Immunostaining was carried out on 6 μm cryostat tissue sections. Tissue sections were air dried for 1 hr at room temperature, fixed in ice-cold acetone for 10 min and then stained by an indirect immunoperoxidase technique as previously described.[21] Briefly, binding of primary monoclonal antibodies (mAb) was detected using peroxidase-conjugated rabbit anti-mouse Ig (Dako Ltd, Copenhagen, Denmark). For smears, sperm were obtained by the swim-up procedure, resuspended at approximately 2×10^6/ml in phosphate-buffered saline (PBS) and, after air drying for 1 hr at room temperature, slides were fixed for 10 min in ice-cold acetone. An enhanced indirect immunoperoxidase technique involving a tertiary incubation step using peroxidase-conjugated swine anti-rabbit Ig (Dako), diluted to 1/60 in Tris-buffered saline (TBS) containing 10% normal human serum, was used to stain sperm preparations.[22] Primary mAb were either used as undiluted tissue culture supernatants or as purified ascites (0·2 μg/ml) diluted in TBS containing 2% (w/v) bovine serum albumin (BSA). Irrelevant primary mAb or TBS alone were included as negative controls.

Antibodies

The following mAb were used: BRIC 110, BRIC 128, BRIC 216, BRIC 220 and BRIC 230 (tissue culture supernatants) against DAF;[21,23] J4-48 (purified ascites, Serotec, Oxford, U.K.) against MCP;[24] BRIC 229 (tissue culture supernatant) against CD59.[25]

Preparation of membranes and solubilization of sperm

Membranes were prepared from testis and prostate specimens by homogenization, on ice, in TBS containing 1 mM phenylmethylsulphonyl fluoride (PMSF). The homogenate was filtered through 100 μm gauze and centrifuged at 300 ***g*** and then 9000 ***g***, for 15 min to remove debris. The resulting supernatant was centrifuged at 100,000 ***g*** for 1 hr at 4° to pellet membranes. The resulting pellet was resuspended in PBS/1 mM PMSF and stored at −70°. Syncytiotrophoblast plasma membranes were prepared from term human placentae by the saline extraction procedure of Smith *et al.*[26] Pellets of sperm cells obtained by the swim-up procedure were resuspended directly in non-reducing Laemmli sample buffer at approximately 1×10^7/ml and solubi-

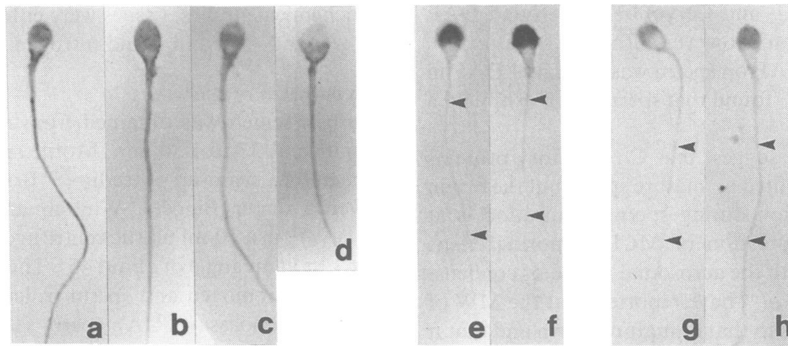

Figure 1. Reactivity of mAb to CD59 (a–d), MCP (e,f) and DAF (g,h) on sperm from a single ejaculate using an enhanced immunoperoxidase staining technique. Anti-CD59 mAb typically stain the acrosomal region, midpiece and tail of sperm (a,b), but in a minority of sperm staining occurs predominantly in the equatorial (c) or post-acrosomal (d) regions with little or no acrosomal reactivity. Monoclonal antibodies to MCP react exclusively with the acrosomal region (e,f); sperm tails (arrowed) are unreactive. Anti-DAF mAb reactivity is located primarily on the acrosomal region (g,h) although there is also weak reactivity with sperm tails (arrows). Note that anti-DAF mAb reactivity on the acrosomal region is weak by comparison with that of mAb to MCP. Magnification ×200.

lized at room temperature for 30 min. Debris was removed by centrifugation at 10,000 ***g*** for 10 min and the extracts were used immediately.

Gel electrophoresis and immunoblotting

Western blotting was carried out using a modification of the method described by Towbin *et al.*[27] Briefly, membrane preparations and solubilized sperm were subjected to SDS–PAGE under non-reducing conditions on gels containing 10% or 12% acrylamide according to the method of Laemmli[28] and electrotransferred to Immobilon-P PVDF membrane (Millipore U.K. Ltd, Watford, U.K.). The membranes were blocked with 5% (w/v) dried bovine milk powder in PBS containing 0·2% (v/v) Tween-20, incubated overnight at 4° with mAb, washed with PBS/Tween-20 and then incubated for 1 hr at room temperature with peroxidase-conjugated rabbit anti-mouse Ig diluted to 1/400 in blocking buffer. The blots were developed in diaminobenzidine and hydrogen peroxide.

Cell-binding assays

Ninety-six-well microtitre plates were blocked with PBS containing 5% (w/v) BSA for 1–2 hr prior to the addition of 1×10^6 sperm/well. Primary mAb incubations were performed in triplicate using either tissue culture supernatants or purified reagents diluted appropriately in TBS supplemented with 5% (w/v) BSA, and binding was detected using ^{125}I sheep anti-mouse F(ab′)$_2$.

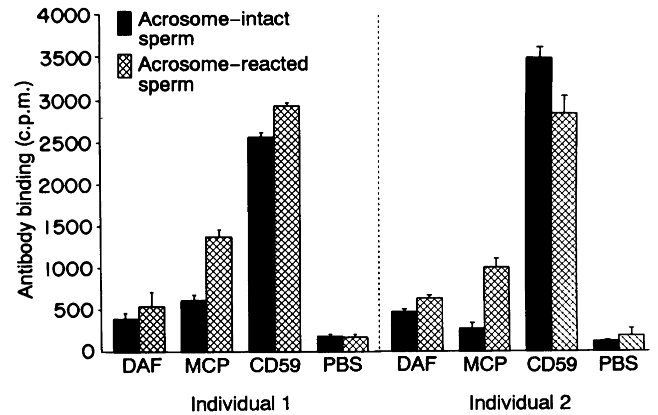

Figure 2. Binding of anti-C regulatory protein mAb to acrosome-intact sperm (prepared by the swim-up technique) and to acrosome-reacted sperm (prepared by the swim-up technique and subsequently ionophore treated) by RIA. Sperm prepared from ejaculates of two individuals are shown. The results are expressed as the means+SE of triplicate observations.

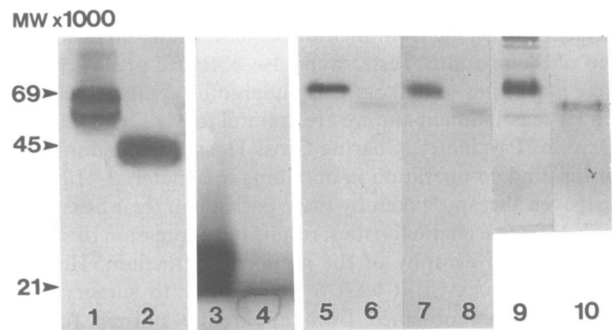

Figure 3. Western analysis of components identified by mAb to MCP (lanes 1 and 2), CD59 (lanes 3 and 4) and DAF (lanes 5–10) on syncytiotrophoblast plasma membranes from term placentae (lanes 1, 3, 5, 7 and 9) and sperm lysates (lanes 2, 4, 6, 8 and 10). The position of MW markers is indicated.

RESULTS

Distribution of C regulatory proteins on sperm

The distribution of CD59, MCP and DAF was examined on swim-up preparations of human sperm by an enhanced immunoperoxidase staining technique (Fig. 1). By comparison with immunofluorescence staining used by previous investigators, we found that this technique improved resolution of mAb localization. In common with previous reports,[11,12] a mAb to CD59 was broadly reactive with both the head and tail regions of sperm. Typically, reactivity occurred on the acrosomal region (Fig. 1a) although we also noted more extensive staining of the head in

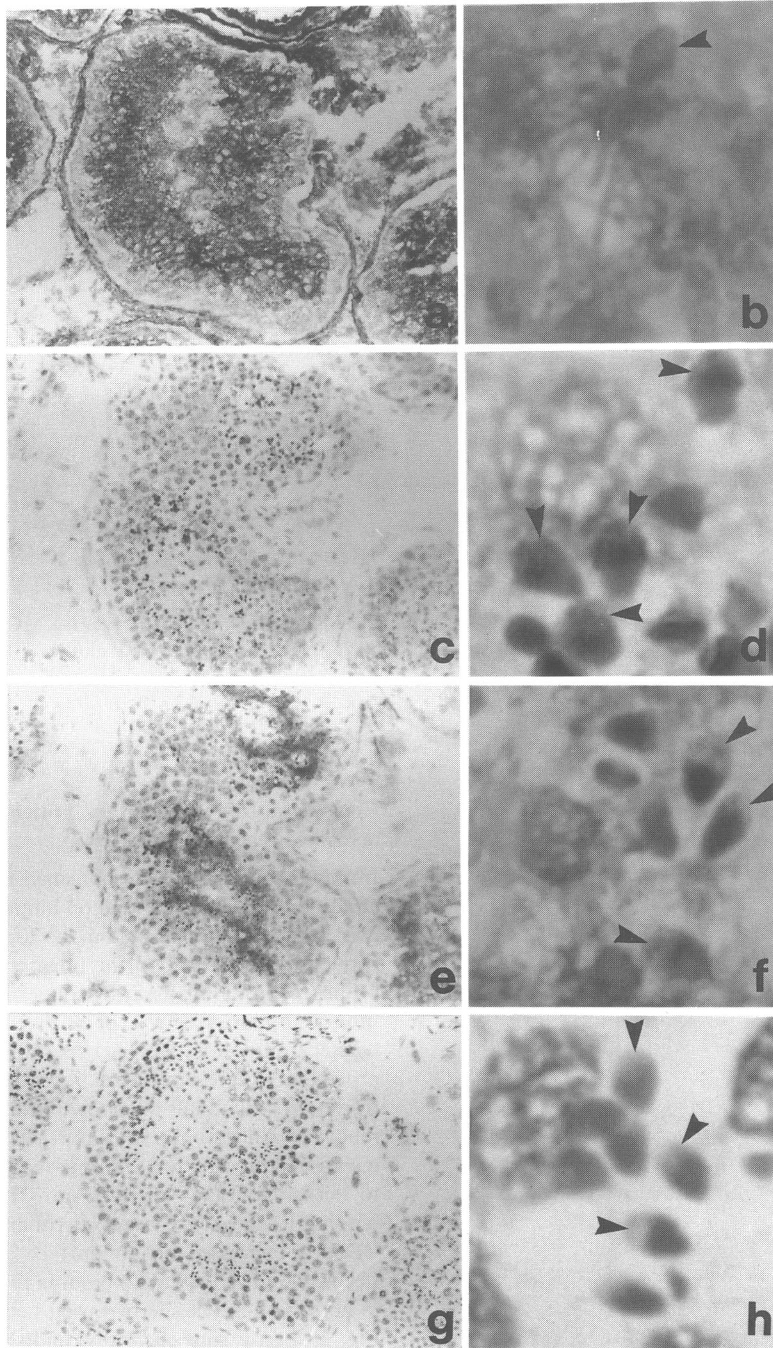

Figure 4. Immunostaining of adjacent sections of normal human adult testis with mAb to CD59 (a,b), MCP (c,d), DAF (e,f) and, in negative controls, with primary mAb omitted (g,h). The general distribution of staining on a single seminiferous tubule is shown at low magnification (× 44) in (a, c, e and g). Reactivity on condensing sperm is shown in more detail (× 440) in (b, d, f and h). Arrows in (b, d, f and h) identify the acrosomal region of condensing sperm.

some sperm (Fig. 1b); intense staining of the midpiece and tail was consistently observed (Fig. 1a,b). Within a single ejaculate, a minority of sperm displayed some variation, exhibiting little or no staining on the acrosomal region (Fig. 1c,d) and pronounced reactivity in the equatorial (Fig. 1c) or post-acrosomal (Fig. 1d) region. It is possible that this may reflect different stages in capacitation or acrosome reaction. The reactivity observed with mAb to MCP was also consistent with previous reports;[9] staining was intense and localized exclusively to the acrosomal region (Fig. 1e,f). In the case of DAF, reactivity also occurred primarily in the acrosomal region, although staining was weak by comparison with anti-MCP mAb (Fig. 1g,h). In contrast to MCP, some weak anti-DAF reactivity was also evident on the tail (Fig. 1g,h).

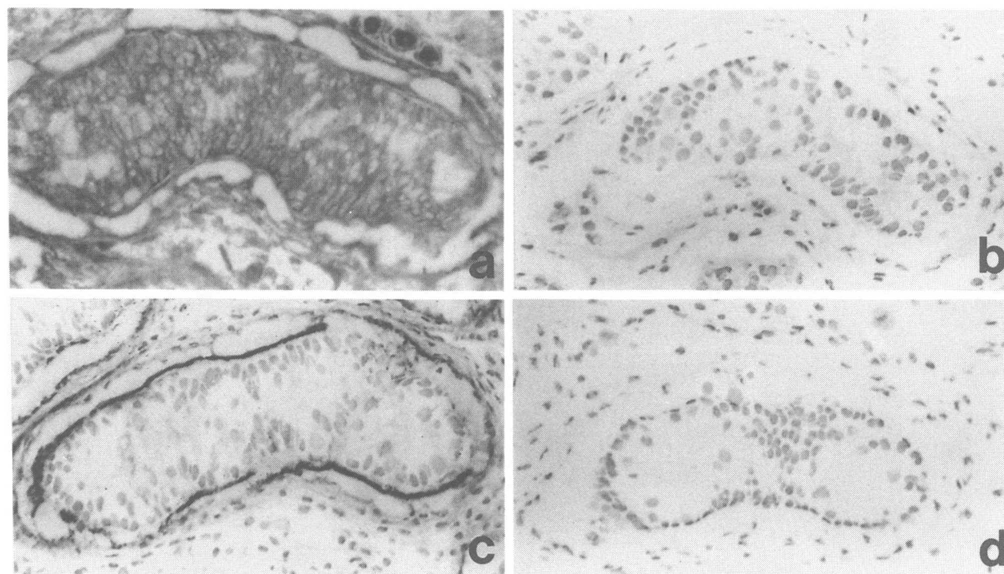

Figure 5. Immunostaining of testis sections from a patient undergoing gender reassignment, with mAb to CD59 (a), MCP (b) and DAF (c). A negative control section (primary antibody omitted) is shown in (d). Magnification ×75. Note the marked irregularities of the basement membrane and diminished germinal epithelium.

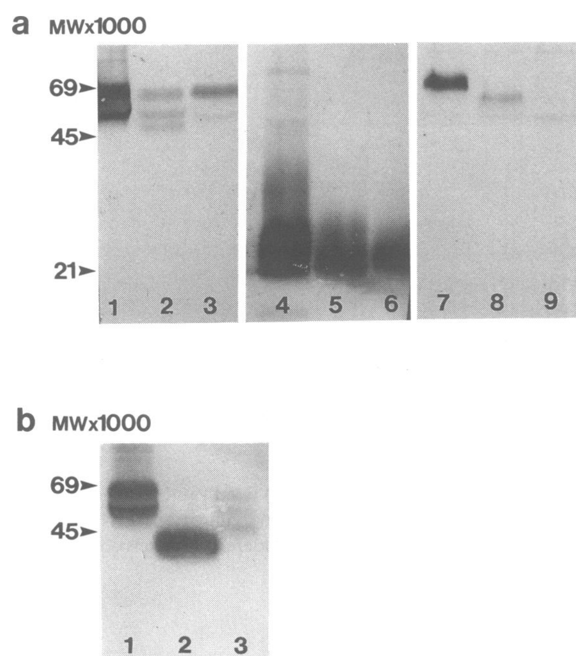

Figure 6. (a) Western analysis of C regulatory proteins detected by mAb to MCP (lanes 1–3), CD59 (lanes 4–6) and DAF (lanes 7–9) on term placental membranes (lanes 1, 4 and 7), normal testis membranes (lanes 2, 5 and 8), and membranes prepared from gender reassignment testis (lanes 3, 6 and 9). (b) Immunoblot comparing the relative MW of components detected by mAb to MCP on sperm (lane 2), normal testis membranes (lane 3) and placental membranes (lane 1). The position of MW markers is indicated.

Comparison of C regulatory proteins on acrosome-intact and acrosome-reacted sperm by RIA

Immunostaining studies confirmed that C regulatory proteins are differentially distributed on human sperm. In an attempt to gain further information on the localization of C regulatory proteins with respect to the inner and outer acrosomal membranes, cell-binding assays were performed on acrosome intact (non-ionophore treated) sperm by comparison with acrosome-reacted (ionophore treated) sperm recovered by the swim-up procedure. Representative experiments conducted on ejaculates from two individuals are shown in Fig. 2. In common with the report of Anderson *et al.*,[9] anti-MCP mAb showed a marked increase in binding to acrosome-reacted sperm when compared to acrosome-intact sperm. Anti-CD59 mAb showed strong binding to acrosome-intact sperm and therefore, unlike MCP, CD59 is expressed on the surface of acrosome-intact sperm. Levels of anti-CD59 mAb binding to acrosome-reacted relative to acrosome-intact sperm varied between individuals. Thus in Fig. 2, sperm from one individual showed an increase in anti-CD59 mAb binding following ionophore treatment while sperm from a second individual showed a reduction in binding. However, binding of anti-CD59 mAb to acrosome-reacted sperm was strong and differences in binding following acrosome reaction were small by comparison with those observed for MCP. Binding of anti-DAF mAb to both acrosome-intact and acrosome-reacted sperm was weak although some increase in binding to acrosome-reacted sperm was evident in both individuals.

Demonstration of C regulatory proteins on sperm by immunoblotting

In order to confirm the expression of C regulatory proteins on sperm, the targets of the panel of mAb were examined by

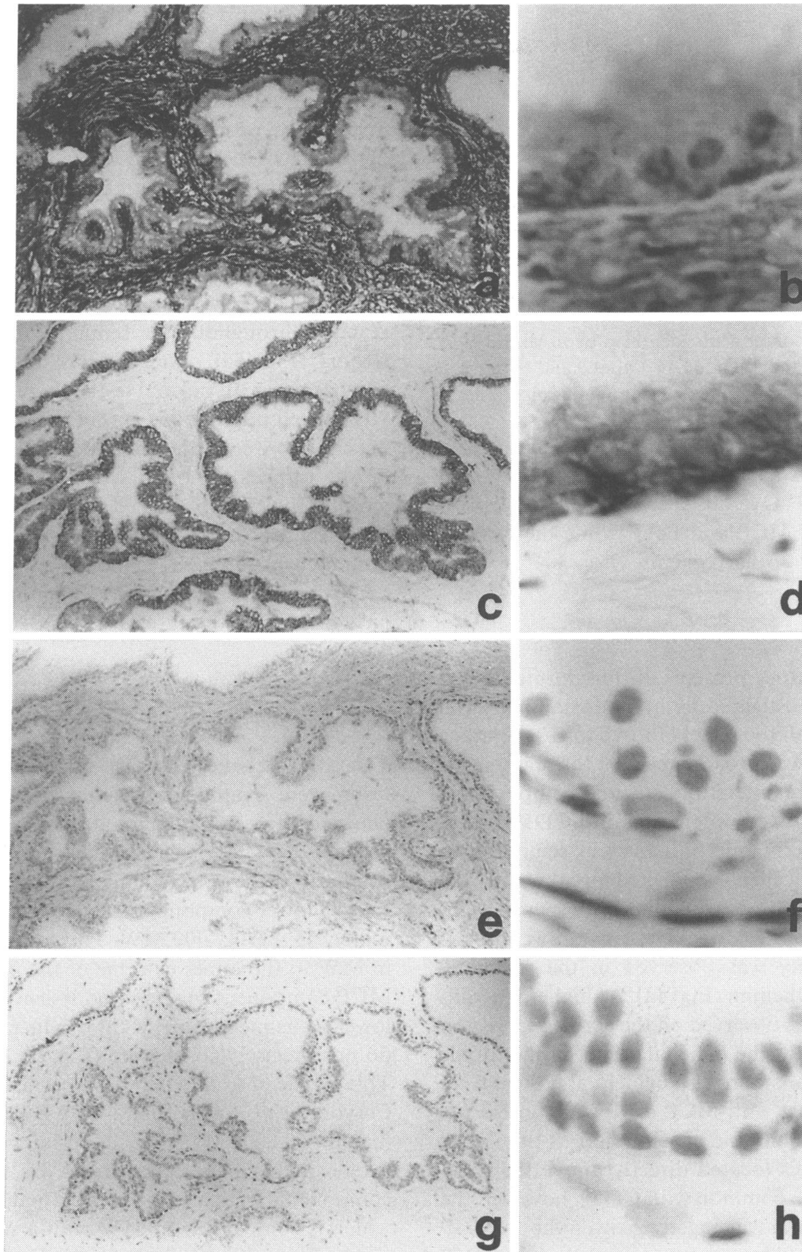

Figure 7. Immunostaining of adjacent sections of human prostate with mAb to CD59 (a, b), MCP (c, d), DAF (e, f), and in the absence of primary antibody (g, h). The general distribution of reactivity on the prostatic epithelium and stromal compartment is shown at low magnification (× 28) on adjacent sections in (a, c, e and g). Reactivity on the glandular prostatic epithelium is shown in more detail (× 280) in (b, d, f and h). Note that the epithelium is reactive with mAb to CD59 and MCP but not with mAb to DAF.

immunoblotting (a, c, e and g) by comparison with targets detected on term placental membranes as these have previously been well documented.[8] Under non-reducing conditions, anti-MCP mAb detected a single, broad band of approximately 40,000–46,000 MW on extracts of human sperm (Fig. 3, lane 2). The component was 10,000–20,000 lower in MW than the two typical MCP components of 50,000–55,000 and 58,000–62,000 MW detected on placental membranes (Fig. 3, lane 1). This migration pattern of sperm MCP is consistent with the report of Cervoni *et al.*[10] An 18,000–20,000 MW protein was detected on extracts of sperm by anti-CD59 mAb (Fig. 3, lane 4). This is consistent with the findings of Rooney *et al.*[11] However in contrast to MCP, the sperm and placental CD59 proteins co-migrated (Fig. 3, lanes 4 and 3, respectively). Bozas *et al.*[12] have reported that the MW of DAF on sperm is typical of that in other systems. In the present study, however, we found that anti-DAF mAb detected a single component of approximately 54,000–57,000 MW on sperm extracts and examples from three individuals are illustrated in Fig. 3 (lanes 6, 8 and 10). DAF on sperm is therefore approximately 15,000 lower in MW than the typical DAF component detected on placental membranes (Fig. 3, lanes 5, 7 and 9). This observation is consistent with the recent

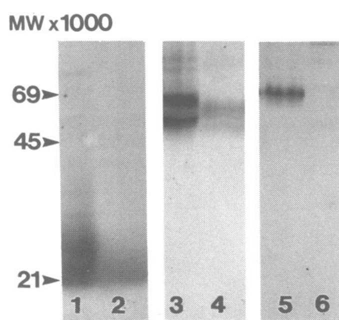

Figure 8. Western analysis of components identified by mAb to CD59 (lanes 1 and 2), MCP (lanes 3 and 4) and DAF (lanes 5 and 6) on term placental membranes (lanes 1, 3 and 5) and prostate membranes (lanes 2, 4 and 6). The position of MW markers is indicated.

report from Cervoni *et al.*[13] Overall, sperm appear to express novel lower MW forms of MCP and DAF while the MW of CD59 on sperm is typical of other systems.

Distribution of C regulatory proteins in human testis

The expression of C regulatory proteins during normal sperm maturation was further investigated by immunostaining serial sections of normal testis with the panel of mAb to C regulatory proteins. Anti-CD59 mAb was broadly reactive throughout the seminiferous tubules and the interstitial stromal compartment (Fig. 4a). However, we observed differential anti-CD59 reactivity within the stratified germinal epithelium of seminiferous tubules. In particular, the cell layer located proximal to the $CD59^+$ basement membrane, which contains both spermatogonia and Sertoli cells, was relatively weakly stained. By comparison, more intense reactivity was observed in the overlying differentiating germinal epithelium (Fig. 4a). At higher magnification (Fig. 4b), staining was observed on the acrosomal region of condensing sperm heads at the lumenal aspect of the tubule (arrow in Fig. 4b). Sperm tails were also evident and these were $CD59^+$ (Fig. 4b). In contrast, anti-MCP mAb were unreactive either with the basement membrane of seminiferous tubules or with germinal epithelial cells located directly above the basement membrane (Fig. 4c). In common with CD59, however, the overlying germinal epithelium was reactive with mAb to MCP although staining was weak by comparison with anti-CD59 mAb (Fig. 4c). Weak staining was also observed in the interstitial stroma. At higher magnification, intense staining of the acrosomal region of condensing sperm heads was evident (arrows in Fig. 4d). Monoclonal antibodies to DAF showed little or no reactivity on germinal epithelium adjacent to the basement membrane although the overlying germinal epithelium was weakly stained (Fig. 4e). In marked contrast to MCP, there was intense anti-DAF reactivity adjacent to the lumen (Fig. 4e). At higher magnification, anti-DAF mAb stained the acrosomal region of condensing sperm heads but staining was weak by comparison with MCP (arrows in Fig. 4f). We also noted some weak anti-DAF reactivity within the basement membrane of some seminiferous tubules and this was particularly evident with mAb BRIC-220 (not shown).

The distribution of C regulatory proteins was also examined in frozen sections of testis from trans-sexual patients who had undergone hormone therapy prior to surgery. These specimens characteristically had a more extensive interstitial stromal compartment and smaller seminiferous tubules by comparison with normal testis. There were marked irregularities of the basement membrane and separation from the peritubular myoid cells, probably reflecting an increase in the width of the peritubular collagen band which frequently occurs in men with seminiferous tubule failure.[29] Although cessation of high-dose hormone therapy at least 6 weeks prior to surgery may allow some regeneration of the germinal epithelium, we observed a diminished epithelium by comparison with normal testis and spermatids were absent (Fig. 5). Anti-CD59 mAb were broadly reactive throughout the seminiferous tubules and interstitial stroma (Fig. 5a) of testis from these patients although, by comparison with normal testis, there was no differential reactivity on cell populations within the seminiferous tubules. Anti-MCP mAb were unreactive with all components of the seminiferous tubules although weak staining was observed in the stroma (Fig. 5b). In marked contrast to the distribution of DAF in the normal testis, anti-DAF mAb were unreactive within the seminiferous tubules (Fig. 5c). We also noted intense anti-DAF reactivity within the basement membrane and this was particularly marked in the case of BRIC-220 (Fig. 5c).

Analysis of C regulatory proteins in human testis by immunoblotting

The characteristics of the C regulatory proteins expressed in testis were examined by immunoblotting. Anti-MCP mAb detected three components on normal testis membranes (Fig. 6a, lane 2). The upper two components migrated with a MW similar to those detected on placental membranes (Fig. 6a, lane 1). The lower component migrated as a sharp band of approximately 45,000–47,000 MW. This lower MCP product is similar in MW to the upper extreme of the broad 40,000–46,000 MW MCP component observed on sperm (Fig. 6b). The sperm and lower testis membrane MCP product are therefore similar but do not precisely co-migrate (Fig. 6b, compare lanes 2 and 3). This profile of testis MCP proteins differs from that reported by Cervoni *et al.*[16] who found only a single product of approximately 40,000 MW which co-migrated with sperm MCP. Interestingly, in a recent report by Seya *et al.*[17] three MCP components were also evident in lysates of human testis, one of which is comparable in MW with a soluble 60,000 MW MCP product found in seminal plasma. However, the MCP products of 60,000–62,000 MW and 54,000–56,000 MW observed in the present study are membrane-associated proteins. The presence of a membrane-associated MCP component of approximately 60,000 MW does not preclude the expression of a soluble protein in the testis.

Immunoblotting of gender reassignment testis membranes revealed only the upper, typical MW MCP components (Fig. 6a, lane 3). These are most likely derived from the stroma as only this compartment, which is expanded in these specimens, was reactive with MCP mAb by immunostaining. The 45,000–47,000 MW MCP component observed in normal testis membranes was absent from gender reassignment specimens suggesting that this protein is normally associated with the developing germinal epithelium or condensing spermatids. CD59 components of 18,000–20,000 MW were detected in both normal and gender reassignment testis membranes (Fig. 6a, lanes 5 and 6) and this appears to reflect the broad distribution of CD59

throughout the stromal and germinal epithelial compartments. Anti-DAF mAb detected a single component of approximately 55,000 MW in normal testis membranes (Fig. 6a, lane 8) and this co-migrated with the DAF product on sperm. DAF components were absent from gender reassignment testis membranes (Fig. 6a, lane 9).

Expression of C regulatory proteins in prostate

It has been shown that sperm may acquire additional CD59 following their interaction with vesicles, designated prostasomes, derived from the prostate.[18] We examined the distribution of CD59 and DAF on prostate by comparison with MCP which is already known to be present at this site.[17] By immunostaining, mAb against all three C regulators stained the stromal compartment although anti-CD59 mAb were more intensely reactive than those against the C3 convertase regulators (Fig. 7, compare a with c and e). By contrast, C regulators appear to be differentially expressed on prostatic glandular epithelium. Monoclonal antibodies to CD59 (Fig. 7a,b) and MCP (Fig. 7c,d) were both strongly reactive and, at high magnification, diffuse staining was observed throughout the epithelium (Fig. 7b,d). The glandular epithelium, however, was unreactive with mAb to DAF (Fig. 7e,f).

By immunoblotting anti-CD59 mAb detected a typical 18,000–20,000 MW component, co-migrating with placental CD59, on membranes prepared from prostate (compare Fig. 8, lanes 1 and 2, respectively). In common with other systems, two MCP components were detected on prostate membranes by immunoblotting (Fig. 8, lane 4). However, the upper and lower MCP bands on prostate each migrated approximately 4,000–5,000 MW ahead of their counterparts on placental membranes (compare Fig. 8, lanes 4 and 3, respectively). Although a typical 70,000 MW DAF product was observed on placental membranes (Fig. 8, lane 5), no DAF components were detected on prostate membranes (Fig. 8, lane 6). This was consistent with the limited reactivity of anti-DAF mAb with the stromal compartment of prostate observed by immunostaining.

DISCUSSION

In this study we set out to compare the distribution of C regulatory proteins CD59, MCP and DAF on sperm and also to examine expression of these proteins during spermatogenesis, and in the prostate. We show that C regulators are differentially expressed on mature sperm and confirm that sperm express a novel 55,000 MW DAF product. In addition, we demonstrate that, although C regulators are also differentially expressed on germinal epithelium in normal testis, all three proteins are associated with condensing spermatids. Thus, the repertoire of membrane-bound regulators of C expressed on mature sperm is acquired during spermatogenesis.

Blocking the functional activity CD59 has been shown to increase the susceptibility of mature sperm to MAC-induced damage *in vitro*[11] and DAF and MCP isolated from sperm have been shown to be functionally active.[10,13] C regulatory proteins have been proposed to play an important physiological role in protecting sperm from C-mediated damage during their transit through the immunocompetent female reproductive tract where functionally active C is present at multiple sites, for example in cervical mucus[15] and ovarian follicular fluid.[14] Notwithstanding this, C regulators are differentially expressed on sperm and this may reflect differences in the requirement for the specific functional activities of the regulators at different locations within the tract.

CD59 is broadly expressed on the head and tail region of sperm and our own binding assay data, together with those previously reported by Rooney *et al.*,[11] suggest that this regulator is expressed on the surface of acrosome-intact sperm. Anti-DAF reactivity was also found to be associated with the tail and acrosomal region of sperm although binding to acrosome intact sperm was very weak. Consistent with Anderson *et al.*,[9] we also found that MCP expression was localized to the inner acrosomal membrane of sperm. Taken together, this suggests that C3 convertase regulators are either weakly expressed or absent from the surface of acrosome-intact sperm despite the potential for initiation of the C pathway particularly in the lower regions of the female reproductive tract. Thus, CD59 appears to be capable of protecting acrosome intact sperm from C-mediated damage in the absence of C3 convertase regulators.

In order for fertilization to occur, sperm must undergo capacitation and acrosome reaction which result in exposure of the inner acrosomal membrane. Expression of MCP at this site suggests that the protein fulfils a role in the upper regions of the female reproductive tract. Interestingly our data, together with those of Cervoni *et al.*,[13] suggest that DAF may also be expressed on this membrane and therefore that the two C3 convertase regulators may act in consort to protect this membrane from C-mediated damage. Thus, while CD59 appears capable of protecting sperm in the lower female reproductive tract, expression of DAF and MCP on the inner acrosomal membrane suggests that there is a specific requirement for protection against C3 convertase formation on this surface which is likely to become exposed in sperm populations approaching the oocyte. Expression of C3 convertases may be important both in preventing access to the terminal C pathway and in regulating the production of pro-inflammatory anaphylotoxins which might compromise fertilization.

In contrast to the observations of Bozas *et al.*,[12] we have found that sperm express a 55,000 MW form of DAF and our data are therefore consistent with the recent report of Cervoni *et al.*[13] Therefore novel low-MW forms of both DAF and MCP are expressed on the inner acrosomal membrane of sperm. Sperm DAF and MCP appear to exhibit alterations in glycosylation by comparison with their counterparts in other tissues.[10,13] The present study reveals that novel MW forms of the C3 convertase regulators are also expressed in testis. Comparison of normal and gender reassignment testis suggests that these products are generated normally within the differentiating germinal epithelium. We also show that, in common with the C3 convertase regulators, CD59 is acquired in the testis and that it is associated with the acrosomal region of spermatids. Nevertheless, in contrast with DAF and MCP, the MW of CD59 on sperm and testis membranes is typical of that observed in other systems. The MW of CD59 is 18,000–20,000 of which, in other tissues, some 4000–6000 is contributed by N-linked carbohydrate. In erythrocytes, this carbohydrate has been demonstrated to

contribute to the functional activity of CD59.[30] This suggests that sperm CD59 may require similar glycosylation to other CD59 antigens in order to maintain functional activity.

CD59, MCP and DAF were all found to be expressed on the acrosomal region of condensing spermatids in normal testis. However, immunostaining studies suggested that there is a differential expression of the regulators on the differentiating germinal epithelium. In particular, CD59 expression in the testis was both more extensive and more intense than that of MCP and DAF. The broad distribution of CD59 at this site is typical of the expression of this protein in many adult[31,32] and developing tissues.[8,33] In addition, the C3 convertase regulators also appear to be differentially distributed in seminiferous tubules; in particular DAF appears to be expressed more intensely than MCP on the generative epithelium adjacent to the lumen. The significance of this is currently unclear although, as DAF products have been described in a number of external secretions,[34] it will be interesting to determine whether such products are present in testis.

In the testis specialized junctional complexes between Sertoli cells are believed to divide the germinal epithelium into a basal compartment, containing spermatogonia, and an adluminal compartment comprising spermatocytes and spermatids. We noted that the layer of cells located proximal to the basement membrane of the seminiferous tubule appeared to express CD59 weakly and was MCP- and DAF-negative. This contrasted with the overlying epithelium which expressed all three regulators. Thus, differentiation of the basal to the adluminal compartment within the germinal epithelium appears to be associated with an increase in the expression of all three C regulators. This may suggest that spermatocytes and spermatids in the adluminal compartment require protection from C-mediated damage.

On leaving the testis sperm enter the epididymis where they are stored. Only at ejaculation are they mixed with secretions from other male accessory glands to form seminal fluid. Bozas *et al.*[12] have recently shown that seminal plasma contains little C3 or C9. The status of other regions of the male reproductive tract, particularly the epididymis, for C components is unknown although it is likely that C is important in controlling infection in this environment. By virtue of their acquisition of C regulatory proteins during spermatogenesis, sperm are protected from C-mediated damage during their transit through the male reproductive tract. It may be speculated that functional C is absent from seminal plasma because additional C components deposited in the female reproductive tract might augment potential damage to sperm in this hostile environment.

Sperm may acquire additional CD59 from prostasomes in seminal plasma.[18] We have shown that the prostatic epithelium, which gives rise to these vesicles, expresses CD59 in addition to MCP but that DAF is absent. DAF may not therefore be present on prostasomes and thus, unlike CD59, this GPI-anchored regulator may be acquired only in the testis. Soluble MCP is present in seminal plasma,[17,35] and although we have identified membrane-bound forms of MCP in prostate, the role of this tissue in production of soluble regulators now needs to be evaluated further.

Overall, we show that C regulatory proteins are differentially expressed on mature sperm and confirm that sperm DAF, like MCP, is a novel low-MW protein. All three regulators are acquired in the testis during normal spermatogenesis and thus sperm leave the testis with the repertoire of regulators required for protection from potential C-mediated damage during transit through both the male and female reproductive tracts.

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