Influence of epididymal maturation on the capacity of hamster and rabbit spermatozoa for complement activation

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Summary. Hamster and rabbit spermatozoa released from the epididymis were tested for the ability to activate complement via the alternative pathway. While hamster spermatozoa were more active, the spermatozoa of both species reduced complement activity in homologous and also human serum previously adsorbed to remove sperm antibodies, and they bound C3 in the presence of EGTA + Mg^{2+} . Hamster spermatozoa from the caput epididymidis were more anticomplementary and bound more C3 than did cauda spermatozoa and, though less marked, a similar difference was evident between caput and cauda spermatozoa from the rabbit epididymis.

Introduction

Internal fertilization has been adopted independently by several different vertebrate groups, but in none is there a clear picture of the complications that insemination may raise for the female, nor of the factors involved in the response to semen within the female tract. The report that bull spermatozoa activate complement via the alternative pathway (Clark & Klebanoff, 1976) suggests a non-immunogenic mechanism that may be one means of sperm removal by the female. Although the extent to which complement components occur throughout the female tract is uncertain (Oliphant, Randall & Cabot, 1977; Schumacher, 1980), haemolytic complement has been detected in cervical secretions (Price & Boettcher, 1979). Furthermore, antibody-coated ova evoke a rapid directed leucocytosis in the oviduct, probably as a consequence of C5a formation, implying the potential for activation of the classical complement pathway at that site (unpublished results). It is also possible that some aspect of change in the sperm surface in the epididymis influences the recognition of and response to spermatozoa by the female tract. We have examined directly the ability of spermatozoa of the rabbit and hamster to activate complement via the alternative pathway, and the effect of epididymal maturation on it.

Materials and Methods

Spermatozoa and serum. Epididymal spermatozoa were obtained from sexually mature male hamsters and rabbits. Human, hamster and rabbit sera containing 0.01 M-EDTA were admixed with spermatozoa from the caput or cauda epididymidis (3.5×10^7 /ml) and incubated for 60 min at 0°C to remove antibodies reactive with spermatozoa. The spermatozoa were then removed by centrifugation and an equal volume of a solution containing 0.02 M-CaCl₂ and 0.005 M-MgCl₂ was added. The treated sera were stored in aliquants at –20°C until used. In pilot experiments, it was

established that results with sera subjected to 2 or more absorptions did not differ from those obtained with sera absorbed only once.

Haemolytic complement activity. Haemolytic complement activity of absorbed sera in the presence or absence of spermatozoa was determined by the method of Palmer (1980). Briefly, absorbed sera were diluted 1:15 in barbitone buffer with or without 6×10^5 spermatozoa (0.3 ml final vol.) and incubated at 37°C for 60 min. The spermatozoa were then removed by centrifugation and the sera serially diluted in barbitone buffer in wells of a round-bottom microfilter plate (0.2 ml/well). Antibody-coated sheep erythrocytes ($2.5 \times 10^6/0.05$ ml) were then added and incubated for 60 min in a water bath at 37°C. The cells were then pelleted by centrifugation and 0.1 ml samples were transferred to a new microtitre plate for measurement of cell lysis by determination of the absorbance at 414 nm. Total haemolytic complement activity (CH_{50}) was calculated as described by Palmer (1980).

C3 binding to spermatozoa. The deposition of C3 from serum onto a cell surface is an indication of complement activation (Pangburn & Muller-Eberhardt, 1978). This was determined here for spermatozoa by an enzyme-linked immunosorbent assay (ELISA). Fresh epididymal spermatozoa, intact as determined by Trypan Blue exclusion (5×10^4 /well for rabbit or 5×10^3 /well for hamster), were fixed to wells of a microtiter plate with 0.5% glutaraldehyde (Witkin, Shahani, Gupta, Good & Day, 1980). The lower concentration of the larger hamster spermatozoa was found to be optimal for their binding to the wells. The fixed spermatozoa were washed three times with PBS containing 0.05% Tween 20 (PBS-Tween) before use. Absorbed human serum was diluted 1:4 in PBS-Tween containing 10 mM EGTA, 2 mM-MgCl₂. EGTA chelates Ca²⁺ in the serum, thereby blocking antibody-mediated (classical pathway) complement activation (Aguado, Perrin, Ramirez, Miescher & Lambert, 1980). The addition of Mg²⁺ permitted only direct (alternative pathway) complement activation by spermatozoa. The sera (0.1 ml) were added to the top row of a microtitre plate and serially diluted with PBS-Tween down the plate. The plate was then incubated in a 37°C bath for 90 min, washed three times with PBS-Tween and each well incubated with 0.1 ml of a 1:200 dilution in PBS-Tween of alkaline phosphatase-conjugated pig IgG antibody to human C3 (Medical Technology Corp., Hackensack, NJ). Following an additional 90 min at 37°C, the wells were again washed three times in PBS-Tween and C3 on the sperm surface was quantitated by the addition of *p*-nitrophenyl phosphate and determination of the absorbance at 405 nm (Witkin *et al.*, 1980). C3 was detected with goat IgG antibody to rabbit C3 (Cappel Labs., Cochranville, PA) when rabbit serum was used, followed by alkaline phosphatase-conjugated rabbit IgG antibody to goat IgG (Litton Bionetics, Kensington, MD).

Results

Effect of epididymal spermatozoa on haemolytic complement activity. Human serum was incubated with spermatozoa from the hamster caput, corpus or cauda epididymidis in 4 separate experiments, and residual haemolytic complement activity was measured (Table 1). Caput spermatozoa were clearly best able and cauda spermatozoa were least able to reduce the haemolytic activity of absorbed human serum. Similar results were obtained regardless of whether the serum had been absorbed previously with cauda or caput spermatozoa, or when cauda-absorbed hamster serum was substituted for human serum.

Similarly, rabbit spermatozoa were incubated in human serum previously absorbed with rabbit cauda epididymal spermatozoa, and haemolytic complement activity was determined in two separate experiments (Table 1). Caput spermatozoa reduced complement activity to the greatest extent, while corpus and cauda spermatozoa of the rabbit were much less effective. Rabbit caput spermatozoa were not as anticomplementary as were hamster caput spermatozoa for human serum on a per cell basis. This may have been due, at least in part, to the larger size of hamster spermatozoa.

Table 1. Effect of spermatozoa from different regions of the epididymis on total haemolytic complement activity (CH_{50})

Species*	Region	Mean CH_{50} (standard deviation)
Blank	—	100
Hamster	Caput	< 10
	Corpus	31 (24)
	Cauda	69 (13)
Rabbit	Caput	61 (19)
	Corpus	93 (5)
	Cauda	103 (6)

Human sera, preadsorbed with hamster or rabbit cauda spermatozoa, were incubated with the various epididymal sperm populations and residual CH_{50} was determined.

* 4 animals for hamster, 2 for rabbit.

Table 2. C3 binding to spermatozoa from different regions of the epididymis

Species*	Region	Mean absorbance (standard deviation)
Hamster	Caput	1.0
	Corpus	0.51 (0.18)
	Cauda	0.27 (0.09)
Rabbit	Caput	1.0
	Corpus	0.63 (0.14)
	Cauda	0.60 (0.23)

Human sera, preadsorbed with hamster or rabbit cauda spermatozoa, were incubated with the various epididymal spermatozoa and C3 binding was measured by ELISA. The values for C3 binding to caput spermatozoa were normalized to an absorbance at 405 nm of 1.0.

* 4 animals for each species.

C3 binding to epididymal spermatozoa. To confirm that hamster caput spermatozoa activate complement to a greater extent than do corpus and cauda spermatozoa, the quantity of C3 deposited on the surface after incubation in serum was measured in 4 separate experiments by ELISA (Table 2). Addition of EGTA and Mg^{2+} to the serum before admixture with the spermatozoa ensured that direct (alternative pathway) complement activation by spermatozoa, and not antibody-mediated activation, was responsible for the C3 deposition. Caput spermatozoa bound the most C3, corpus spermatozoa an intermediate amount, and cauda spermatozoa bound the least amount of C3 at the cell surface.

Human and rabbit sera previously absorbed against rabbit cauda spermatozoa were also incubated with rabbit spermatozoa from the successive regions of the epididymis, and C3 binding was determined in 4 experiments. As with hamster spermatozoa, rabbit caput spermatozoa always bound more C3 than did corpus and cauda spermatozoa (Table 2), and this decrease in binding of C3 to the sperm surface with maturation was qualitatively unaffected by chelation of Ca^{2+} .

Discussion

It has been proposed that immunoglobulin binds to spermatozoa in the epididymis (Suarez, Hinton & Oliphant, 1981) and that this association eventually results in classical pathway complement

activation in the female tract, thereby initiating the acrosome reaction (Suarez & Oliphant, 1982). It is highly unlikely that sperm-induced complement activation serves this function. Antibody-coated spermatozoa are generally unable to traverse cervical mucus (Jager *et al.*, 1981) or avoid complement-mediated immune destruction (Bronson, Cooper & Rosenfeld, 1982) in the cervix or uterus. In addition, living spermatozoa can undergo the acrosome reaction in a defined medium *in vitro* (Rogers, 1978) in the absence of complement, and in complement-depleted females *in vivo* (Bedford & Witkin, 1983). Regardless of whether immunoglobulin may be associated with epididymal spermatozoa, our results confirm for hamster and rabbit the discovery by Clark & Klebanoff (1976) that bull spermatozoa directly activate complement via the alternative pathway without the participation of specific antibody. The level of that ability is not a fixed characteristic, but diminishes as a function of epididymal maturation. That complement depletion permits more spermatozoa to populate the upper reaches of the reproductive tract in the rabbit, in which insemination is vaginal (Bedford & Witkin, 1983), points to this ability as one possible element for their fate in the female. As it appears now, the plasmalemma of the mature spermatozoon would have the least tendency to activate complement and so may have a selective advantage in avoiding immune destruction.

Which intrinsic constituents of the sperm surface activate complement are not known. It is likely, however, that the diminished ability for complement activation reflects some aspect of epididymal surface change (Moore, 1981; Feuchter, Vernon & Eddy, 1981). It is tempting to consider sialic acid groups as being important, since these moieties minimise the tendency of certain other cells to activate complement via the alternative pathway (Fearon, 1978). However, the status of sialic acid in relation to epididymal maturation surface changes is presently unclear (Holt, 1980; Toowicharanont & Chulavatnatol, 1983). It is possible that, according to species, other terminal side chains of sperm surface glycoproteins, as well as *n*-acetyl neuraminic acid, may specifically diminish the ability of the sperm surface to activate complement.

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