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Proteasomes in human spermatozoa

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Summary

In the present study we describe the localization of proteasomes in human spermatozoa by means of immunolabelling with different monoclonal and polyclonal antibodies detected by confocal microscopy. Western blotting confirmed the specificity of the antibodies and has shown that proteasomes are present in spermatozoa and in seminal fluid. In spermatozoa proteasomes are concentrated in the neck region where the centrioles are located. Some labelling was also detected at the periphery of the head, but no proteasomal antigens were detected in either the nucleus or associated with the flagellum. Proteasome inhibitors did not affect the motility of the spermatozoa, acrosome reaction nor zona binding. It is hypothesized that paternal proteasomes enter the oocyte during fertilization in tight association with the centrioles and may serve a special function during further development which can be associated with the function of a hypothetical proteolysis centre.

Keywords: proteasome, proteasome activator, proteolytic centre, spermatozoa

Introduction

Ubiquitin and proteasome-mediated proteolysis is responsible for the degradation of most cell proteins (Rock *et al*., 1994). The 20S proteasome is an ~700 kDa complex, formed by four stacked rings surrounding inner cavities: two inner β rings and two outer α rings. The rings of each type are composed of seven different subunits of the α and β family, respectively (Zwickl *et al*., 1992; Coux *et al*., 1996; Tanaka, 1998). The 20S proteasome is a multicatalytic proteinase with hydrolytic activities defined against small synthetic peptides as chymotrypsin-like (ChTL), trypsin-like (TL), peptidylglutamylpeptide hydrolysing (PGPH), small neutral amino acids preferring (SNAAP), and branched chain amino acid preferring activity (BrAAP) (Orlowski, 1990). Free N-terminal threonine (Thr) residues on three of the seven β-type subunits act as nucleophiles and are essential in the mechanism of catalysis (Fenteany *et al*., 1995; Seemuller *et al*., 1995). In contrast to lower eukaryotes, the mammalian genome encodes six active β subunits, despite the fact that each proteasome contains only three of them. These subunits are exchanged after interferon γ stimulation (Tanaka & Kasahara, 1998). Various inhibitors of the proteasome have been developed, among them the peptidyl aldehyde PSI and the antibiotic lactacystin (Lee & Goldberg, 1998; Wojcik, 1999).

The 20S proteasomes degrade only small peptides because the access to their catalytic chamber is obstructed by the N-termini of the α subunits (Groll *et al*., 1997). Additional protein complexes or proteasome activators must associate with the 20S proteasome, presumably facilitating the access to the catalytic chamber. Ubiquitinated proteins are degraded by 26S proteasomes of ~2000 kDa, formed by association of the 20S proteasomes with the PA700 activator (19S cap). Other activators, such as PA28(α,β) or PA28γ (Ki antigen) activate only the hydrolysis of small peptides (Coux *et al*., 1996; Tanaka, 1998). Hybrid proteasome complexes

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containing both PA700 and PA28 have also been described (Hendil *et al.*, 1998).

The 26S proteasomes degrade proteins which have been recognised and tagged with the polyubiquitin chain by a cascade of enzymes, consisting of ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin ligase (E3). Ubiquitin is added, forming an isopeptide bond between the ε-amino group of an internal Lys of the substrate and the C-terminal Gly of ubiquitin. As ubiquitin itself contains internal Lys residues, additional ubiquitin moieties can be added by the same mechanism (usually to Lys48) (Varshavsky, 1997; Ciechanover & Schwartz, 1998; Tanaka, 1998).

Proteasomes have been detected in all eukaryotic cells studied to date, and also in the gametes of numerous species. In sea urchins proteasomes are involved in the acrosome reaction (Matsumura & Aketa, 1991), while in ascidians sperm proteasomes take part in the fertilization process (Saitoh *et al.*, 1993) since proteasome inhibitors block fertilization (Takizawa *et al.*, 1993). Proteasomes are present in salmon spermatozoa along the axoneme (Inaba & Morisawa, 1992; Inaba *et al.*, 1993), and apparently perform some function related to their motility because proteasome inhibitors immobilise the spermatozoa (Inaba *et al.*, 1993). It is well established that sperm motility is regulated through cAMP levels. In fish, proteasomes regulate cAMP-dependent phosphorylation of the 22 kDa dynein light chain present in the axoneme (Inaba *et al.*, 1998).

Proteasomes have been purified from mouse and human spermatozoa (Tipler *et al.*, 1997). A subunit of the 26S proteasome has been shown to be associated with paraaxonemal mitochondria and the outer dense fibres of the developing spermatid tail in rats (Rivkin *et al.*, 1997). Despite these reports, no study has been published on the localization and function of proteasomes in mature mammalian spermatozoa.

In this paper we present data showing the localization of proteasomal antigens in human spermatozoa. We also show that proteasome inhibitors do not affect sperm motility, acrosome reaction and zona binding. A survey of expression of proteasome subunits in the semen of various patients with male infertility has not shown any correlation between the detection of proteasomal antigens and the pathological conditions, despite the fact that the proteasome immunoreactivity roughly corresponds to the concentration of spermatozoa in semen.

Materials and methods

Reagents and antibodies

Reagents. Ham's F10 medium was from Gibco Life Technologies Ltd. (Paisley, Scotland, UK), IVF medium was from Medi-Cult (Copenhagen, Denmark), propidium iodide was from Boehringer Mannheim (Mannheim, Germany), and Yo-Pro™-1-iodide was from Molecular Probes (Eugene, OR, USA). PSI (N-benzyloxycarbonyl-Ile-Glu(O-*t*-Bu)-Ala-Leucinal) was a generous gift from Dr Sherwin Wilk (Mount Sinai School of Medicine, NY, USA). It was synthesised as described by Figueiredo-Pereira *et al.* (1994), and kept as a 5-mM stock solution in DMSO at −20 °C until further use. Lactacystin purified from *Streptomyces* sp. was a generous gift from Dr Satoshi Omura (Kitasato Institute, Tokyo, Japan) (Omura *et al.*, 1991), and also kept as a 5-mM stock solution in DMSO at −20 °C until further use. All the remaining reagents were from Sigma (St. Louis, MO, USA).

Antibodies. MCP21, MCP34, antidelta, and p45–32 monoclonal antibodies were a kind gift from Dr Klavs. Hendil (August Krogh Institute, University of Copenhagen, Copenhagen, Denmark) (Kaltoft *et al.*, 1992), anti-LMP7 serum was a generous gift from Dr Sherwin Wilk (Mount Sinai School of Medicine, NY, USA) (Wojcik & Wilk, 1999), anti-PA28-α, -β and -γ sera were kindly provided by Dr Keiji Tanaka (Tokyo Metropolitan Institute for Medical Studies, Tokushima, Japan) (Tanahashi *et al.*, 1997). MCP21 detects the HC3 proteasome subunit, while MCP34 detects the XAPC7 proteasome subunit, both of the α-type. Anti-delta monoclonal antibody and anti-LMP7 serum detect the catalytically active β-type subunits, delta (Y) and LMP7, respectively. The p45–32 antibody detects the p45 ATPase (Sug1 homologue) of the PA700 activator complex, while the anti-PA28-α, -β and -γ sera detect the respective subunits of the PA28 proteasome activator.

Secondary antibodies used for Western blotting were alkaline phosphatase conjugated antirabbit and antimouse Fab′ fragments from Jackson Immunoresearch (West Grove, PA, USA). Secondary antibodies used for immunofluorescent labelling were Cy™ 3-conjugated donkey antimouse AffiniPure IgG (H + L) from Jackson Immunoresearch (West Grove, PA, USA) or Alexa™ 488-conjugated goat antirabbit IgG (H + L) from Molecular Probes (Eugene, OR, USA). The molecular size markers used were the prestained kaleidoscope from BioRad (Hercules, CA, USA).

Collection of semen samples

Semen samples were obtained by masturbation from patients undergoing routine semen analysis at the infertility clinic in Hospital Edouard Herriot, Lyon, France. After liquefaction, semen was centrifuged at 1100 ***g*** for 10 min in a Jouan C1000 S5L centrifuge, and the seminal fluid was removed, while the pellet was resuspended in PBS, and centrifuged again for 5 min at 500 ***g***. For Western blotting, samples were collected from whole semen, seminal fluid or purified spermatozoa by suspension and boiling in SDS-PAGE sample buffer (pH 6.8, 0.76% Tris, 2% SDS, 10% glycerol, 1% mercaptoethanol, 0.003% bromophenol blue). A double volume of sample buffer was used for each volume of whole semen or seminal fluid, while the pellet obtained

from 2 mL of semen was suspended in 200 μL of sample buffer. Samples were stored frozen at −20 °C for a maximum of one month before SDS-PAGE.

For motility analysis and immunofluorescence labelling, motile spermatozoa were selected by centrifugation through a discontinuous Percoll gradient (Berger *et al.*, 1985). Briefly, 1 mL semen was carefully layered over a 50%/70%/90% gradient of Percoll and centrifuged for 20 min at 350 ***g*** in a Jouan C1000 S5L centrifuge. The pellet was resuspended in 5 mL Ham's F10 nutrient mixture, and centrifuged again for 10 min at 500 ***g*** then resuspended in an appropriate volume of IVF medium (Medicult, Denmark) and used for further procedures.

SDS-PAGE and Western blotting

Sperm samples were heated by short immersion in boiling water and run on 12% SDS-PAGE in a vertical slab gel unit (Owl Scientific Inc., Woburn, MA, USA) (Laemmli, 1970). After SDS-PAGE the gel was blotted by a semidry method using the isotachoforetic buffer system with The Panther™ electroblotter (Owl Scientific Inc., Woburn, MA, USA) on the PVDF transfer membrane (Biorad, Hercules, CA, USA). After transfer, the membrane was blocked (5% dry skimmed milk, 2% BSA, 0.2% Tween-20 in TBS), washed three times, incubated with primary antibody, washed three times again, and incubated with secondary antibody conjugate. Alkaline phosphatase detection was carried out using standard freshly made BCIP/NBT substrate mixture (Sambrook *et al.*, 1989). The reaction was stopped by addition of distilled water and the images were scanned.

Immunofluorescence confocal microscopy

Motile spermatozoa obtained after Percoll gradient centrifugation were spread on a slide and air dried for 30 min before fixation in freshly made 2% formaldehyde in PBS. In order to avoid misinterpretation as a result of individual variation, the spermatozoa were obtained from a mixture of semen obtained from four individuals without semen abnormalities as determined by routine laboratory analysis according to the WHO criteria (World Health Organization, 1999). Cells were then quenched in 50 mM NH_4Cl, permeabilized in 0.1% Triton X-100 and washed twice in TBS (Tris-buffered saline, pH 7.6) supplemented with 1% BSA and 1% fish skin gelatine, before incubation with the first antibody. After extensive washing, the slides were incubated with the secondary antibody and washed in TBS. Slides were treated with RNAse for 15 min and counterstained with propidium iodide or Yo-Pro™-1-iodide. Finally the slides were embedded in fluorescence mounting medium (Dako Corporation, Carpinteria, CA, USA).

After mounting, samples were kept for up to one week at 4 °C in darkness before examination with an LSM 10 confocal laser scanning microscope (Zeiss, Oberkochen, Germany). The excitation source was a 488-nm wavelength Argon laser, and fluorescence emission was recorded by two detectors situated behind a 590-nm long pass, a filter and a 515–545 nm band pass. Both excitation and emission light were focused through a ×40 Plan-Neofluar immersion objective with 1.3 numerical aperture and an additional electronic zoom produced the definitive images. Optical sections were acquired, each one with an image resolution of 512 × 512 pixels.

Analysis of sperm motility

Percoll selected spermatozoa were incubated in IVF medium either with 50 μM PSI, 50 μM lactacystin or with 2% DMSO as a control at 37 °C under a humid atmosphere containing 5% CO_2. At different time intervals 4 μL samples were placed in 20 μL MicroCell chambers (Conception Technologies, San Diego, CA, USA), then computer-assisted semen analysis (CASA) was performed using an ATS 20 system (JC Diffusion International, Vimontiers, France), which analysed main sperm motility characteristics (curvilinear velocity, straight line velocity, amplitude of lateral displacement). For each evaluation at least 150 motile spermatozoa were selected.

Zona binding assay

Oocytes which failed to develop for the two days following a routine ICSI procedure were placed in fresh IVF medium containing either 100 μM PSI or 2% DMSO as a control in a culture well. After careful microscopic inspection for the lack of zona-attached spermatozoa, a suspension of Percoll purified motile spermatozoa was added containing at least 100 000 spermatozoa. Subsequently, the culture dishes were placed at 37 °C in humid atmosphere containing 5% CO_2. After 4 h the oocytes were carefully inspected for the presence of attached spermatozoa. The binding assay was considered positive, when numerous spermatozoa were found attached to the surface of the zona pellucida.

Acrosome reaction assay

Percoll purified spermatozoa were preincubated for 1 h either in control IVF medium or in the same medium containing 50 μM PSI at 37 °C, and a humidified atmosphere enriched with 5% CO_2. The calcium ionophore A23187 (Sigma) from a 10-mM stock solution in DMSO was added to give a final concentration of 100 μM, and the incubation was continued for another 1 h. The spermatozoa were then fixed in 4% paraformaldehyde in PBS, washed in 100 mM ammonium acetate (pH 9.0), stained with Coomassie blue (Larson & Miller, 1999), air dried, embedded in mounting medium and observed in a microscope at 400× magnification.

Results

SDS-PAGE and Western blotting

In order to determine the presence of proteasomal antigens in spermatozoa, lysates prepared from whole semen,

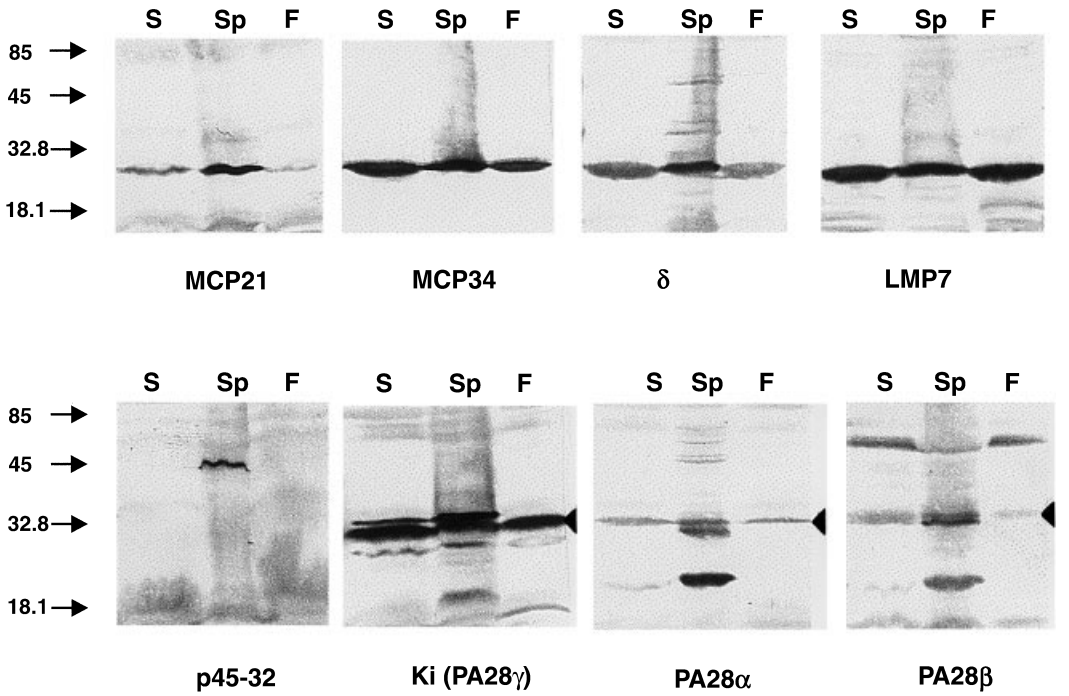

Figure 1. Western blotting of lysates obtained from whole semen (S), purified spermatozoa (Sp) and seminal fluid (F) using the MCP21, MCP34 and antiδ monoclonal antibodies as well as the polyclonal anti-LMP7 serum (detecting different 20 S proteasome subunits). The p45–32 monoclonal antibody detected the p45 ATPase of the PA700 proteasome activator, and polyclonal antisera detected the proteasome activator PA28γ (Ki antigen) and the proteasome activator PA28 subunits α and β. In the case of the latter three antigens various bands are seen on the blots, and arrowheads point to the band of expected molecular size corresponding to the antigens of interest (all of ~30 kDa). The position of molecular size markers are indicated on the left.

as well as from seminal fluid, and purified spermatozoa were subjected to one-dimensional SDS-PAGE. The samples for the initial detection of proteasome antigens were obtained from a mixture of semen obtained from four individuals without semen abnormalities as determined by routine laboratory analysis.

Proteasomes were detected in whole semen and purified spermatozoa, as well as in seminal fluid (Fig. 1). Monoclonal antibodies MCP21 and MCP34 detected single bands of the expected molecular size, while the anti-LMP7 polyclonal antiserum and antidelta monoclonal antibody detected a major band of ~22 kDa and some minor bands (Fig. 1, upper panel). In the case of MCP21 and antidelta monoclonal antibodies the intensity of the bands was lower in seminal fluid and whole semen than in purified spermatozoa.

The p45 ATPase of the PA700 proteasome activator complex (19S cap) was detected only as a weak band in the sample of purified spermatozoa, with the use of p45–32 monoclonal antibody, while it was not detected either in whole semen or in purified seminal fluid. The two subunits of the PA28 proteasome activator (α and β) as well as the related PA28γ (Ki antigen) were detected in whole semen, seminal fluid and purified spermatozoa. While the anti-Ki serum reacted mainly with a major band of the expected molecular size of ~30 kDa, the anti-PA28α and anti-PA28β sera detected also multiple bands beside the major bands of ~30 kDa. It was particularly noticeable that the anti-PA28α serum reacted with a protein of ~20 kDa present in spermatozoa (Fig. 1, lower panel).

In order to check, whether individual variations in the expression of proteasomal antigens could occur, semen samples of patients with different semen abnormalities (including asthenospermia and teratospermia) were also subjected to SDS-PAGE and Western blotted (not shown). In all cases we obtained a similar pattern of expression as described above, with the exception that the bands were weaker in cases of oligospermia.

Immunofluorescence confocal microscopy

The use of MCP21 monoclonal antibody and anti-LMP7 serum for immunofluorescence labelling of spermatozoa revealed that both antibodies label apparently the same structures in the neck region of the spermatozoa (Fig. 2c,f). In most cases a clear, bright spot was observed in the position of the sperm neck. Usually also some fine labelling was detected at the periphery of the head, revealing some discrete pattern of proteasome distribution. In some cases the posterior region of the head was marked, while in other cases the anterior region or the border between the two was labelled. When cytoplasmic fragments were found, they usually were heavily immunolabelled. No other structures of the spermatozoa were labelled. With the use of the anti-Ki

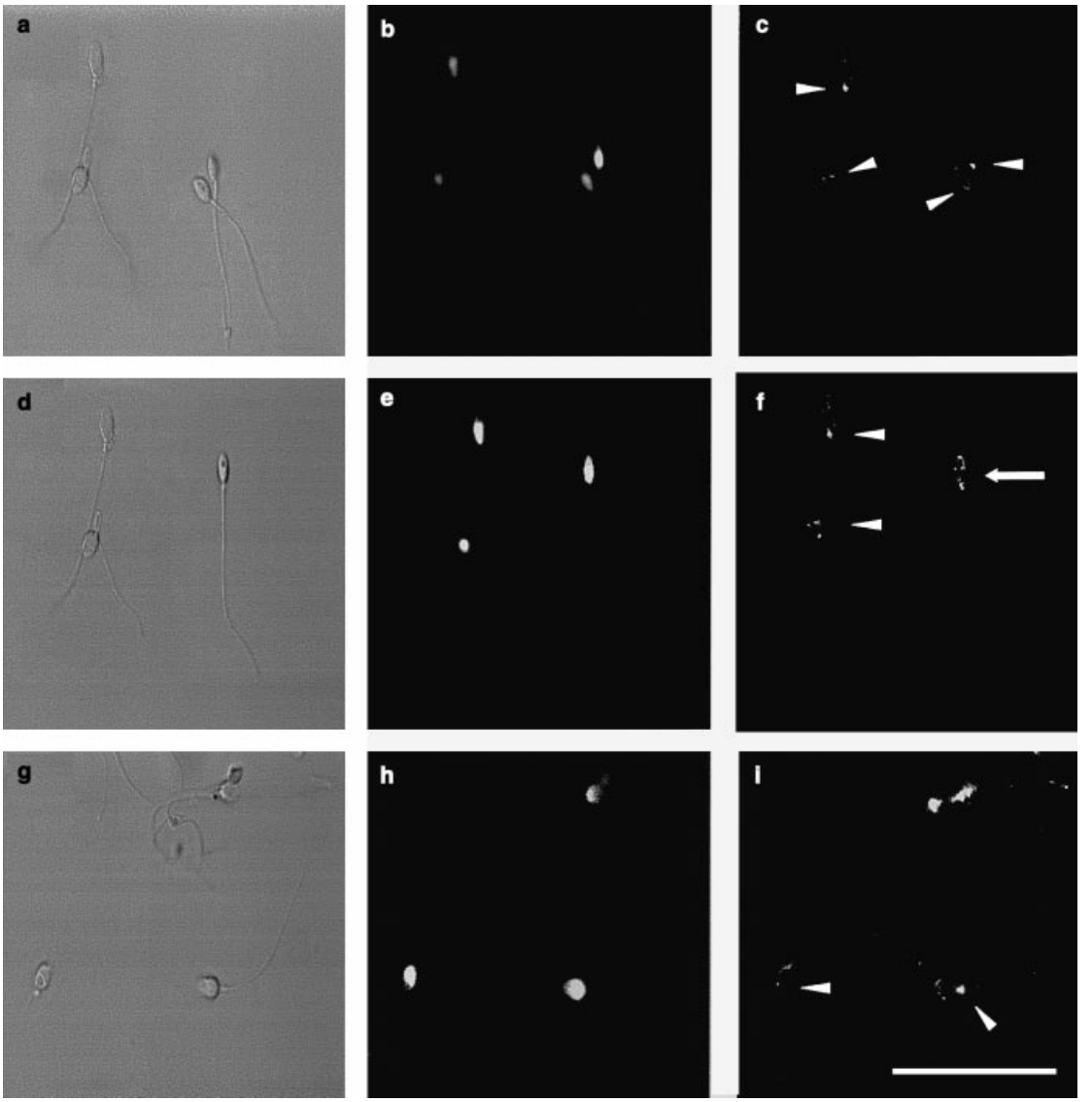

Figure 2. Localization of proteasome-related antigens on spermatozoa using confocal laser scanning microscopy. In each row, the same specimen is viewed under phase contrast illumination (a, d, g) or as a confocal section with DNA labelled with YoPro™-1-iodide (b, h) or propidium iodide (e) and immunolabelled with the antibodies MCP21 (c), anti-LMP7 (f), and anti-PA28γ (i). Arrowheads on (c, f, i) point to strong immunolabelling of the necks in individual spermatozoa, while the arrow in (f) points to a spermatozoon with both the neck and the acrosome region labelled. Bar indicates 30 μm.

(PA28γ) serum we detected a similar pattern of labelling (Fig. 2i) of the neck and the head regions. Control experiments using nonimmune rabbit and mouse sera revealed only very weak background fluorescence, with intensity too low to be scanned by the confocal microscope.

Motility assay, acrosome reaction assay and zona binding assay

CASA revealed no significant differences between PSI or lactacystin treated and control spermatozoa, both in terms of the percentage of motile spermatozoa and the classical sperm movement characteristics (as mentioned in Material and methods). In the control group the percentages of sperm motility were 83, 71 and 47%, vs. 71, 79 and 38% in the presence of 50 μM PSI, at 2, 4 and 24 h, respectively.

There was no significant difference between the percentage of acrosome reacted spermatozoa between control preparations, also in the presence of 50 μM PSI. In both cases 100 μM calcium ionophore A 23187 induced 79% of acrosome reacted spermatozoa, compared with 12% in control nontreated spermatozoa and 16% in PSI preincubated spermatozoa.

The zona binding assay was positive in all cases studied. Microscopical inspection revealed that spermatozoa bound in high numbers to the zonae of nonfertilized post-ICSI oocytes regardless of the presence of 50 μM PSI in the

incubation medium. No differences were noticed between the control and treated group.

Discussion

Our report is the first to show the localization of proteasomes and their activators in human spermatozoa. Proteasomes from human spermatozoa have been purified and characterized biochemically by Tipler *et al.* (1997). The above mentioned authors have shown that sperm proteasomes share similar sedimentation coefficients, peptidase activities and presence of 20S proteasome subunits with 26S proteasomes purified from other human tissues (Tipler *et al.*, 1997).

In our studies we used a panel of monoclonal antibodies and polyclonal sera raised against various subunits of the proteasome and its activators. The initial characterization of these antibodies by Western blotting of sperm lysates let us select, for the immunocytochemical studies, the MCP21 monoclonal antibody which detected a single band of the correct molecular size, and the anti-LMP7 serum which reacted mainly with a single band of the correct molecular size, with some minor binds of a lower molecular size. We also used the anti-Ki (PA28γ) antiserum, but because it detected various bands on the Western blots, the immunocytochemical results obtained with it should be interpreted with great caution and only in the context of the first two antibodies.

During spermiogenesis the ubiquitin-proteasome system mediates the degradation of numerous proteins. The more noticeable example is the degradation of histones, which are replaced by protamines, leading to chromatin compacting. The loss of a specific ubiquitin-conjugating enzyme HR6B leads to male infertility in mice (Roest *et al.*, 1996; Baarends *et al.*, 1999). It is difficult however, to conceive a proteolytic process requiring the ubiquitin-proteasome system in mature spermatozoa. It is not surprising, therefore, that in mice mature spermatozoa have considerably reduced amounts of a ubiquitin-conjugating enzyme (E2), and ubiquitin-protein conjugates in comparison with less mature germ cells. However, they retain relatively high proteasome activity (Tipler *et al.*, 1997). We confirmed these findings by showing that spermatozoa contain various subunits of the 20S proteasome, an ATPase subunit of PA700 activator (p45–32) and PA28γ (Ki antigen), α and β. With the exception of p45, we detected all of these antigens not only in the spermatozoa, but also in seminal fluid. Since 20S proteasomes are intracellular organelles (Rubin & Finley, 1995; Coux *et al.*, 1996; Tanaka, 1998), their presence in the seminal fluid probably reflects their liberation from lysed cells.

We found, that both the constitutive δ and the interferon-γ inducible LMP7 catalytically active subunits are expressed in spermatozoa at comparable levels. Cells of the spermatogenic lineage do not express interferon-γ, however, it is produced in high amounts in Leydig cells which may play a role in antiviral defence (Dejucq *et al.*, 1998), and is present in seminal fluid (Fujisawa *et al.*, 1998).

As judged from the immunolabelling with MCP21 and anti-LMP7 antisera, the proteasomes are present in the neck of spermatozoa, where the centrioles are located. The PA28γ (Ki antigen) proteasome activator also has a similar localization. Due to low immunoreactivity, we were not able to use the p45–32 antibody for immunolabelling. Some proteasomes and PA28γ are also present in the periphery of the sperm head, around the nucleus, at the acrosome or at the presumptive border between the acrosome and posterior part of the head. No labelling was found in the middle piece or the remainder of the flagellum. This finding contrasts with the localization of proteasomes in salmon spermatozoa, where they were detected along the axoneme (Inaba & Morisawa, 1992; Inaba *et al.*, 1993).

The fact that proteasomes were not detected inside the sperm nucleus is in striking contrast with somatic cells, where proteasomes are usually detected in nuclei with the exception of the nucleoli (Rivett *et al.*, 1992; Amsterdam *et al.*, 1993; Wojcik *et al.*, 1995). Blastomeres derived from fragmented human embryos also exhibit strong nuclear labelling with antiproteasome antibodies (Wojcik *et al.* 2000). We therefore propose that the chromatin remodelling during spermatogenesis probably also involves export of nuclear proteasomes. The observed situation resembles that observed in mitotic cells, where proteasomes are not associated with chromosomes but dispersed in the cytoplasm and associated with the mitotic apparatus (Kawahara & Yokosawa, 1992; Amsterdam *et al.*, 1993; Wojcik *et al.*, 1995). An alternative explanation for the lack of nuclear staining could be that it is an artefact due to the difficulty of the antibodies to penetrate inside the compact structure of the sperm nucleus despite Triton permeabilisation.

Proteasome inhibitors were reported to inhibit the motility of salmon spermatozoa (Inaba *et al.*, 1998). However, two cell-permeable, potent and relatively specific proteasome inhibitors, PSI (Figueiredo-Pereira *et al.*, 1994) and lactacystin (Fenteany *et al.*, 1995), used in high concentrations and for a long time, did not affect the mobility of human spermatozoa at all. This finding is consistent with the different localization of proteasomes in human vs. fish spermatozoa as indicated in the present study. Nevertheless, a possibility remains that sperm membranes are absolutely impermeable to these inhibitors.

In sea urchins, proteasomes are involved in the acrosome reaction (Matsumura & Aketa, 1991). Therefore, we decided to check whether PSI would interfere with the acrosome reaction elicited by high concentration of the calcium ionophore A 23187. We observed no differences in the amount of acrosome reacted spermatozoa between control and PSI treated spermatozoa. However, because we used a rather unphysiological stimulus and detected some proteasome immunolabelling at the level of the acrosome, the

possibility that proteasomes can somehow contribute to the acrosome reaction under physiological conditions cannot definitively be ruled out. We have also shown that proteasome inhibition by PSI does not interfere with zona binding.

Because proteasome inhibitors seem not to affect the normal functions of spermatozoa and proteasomes are present in high concentration in the neck of the spermatozoa, the question arises as to what their function could be. We speculate that sperm proteasomes are only stored in this location and do not perform any function until fertilization occurs. It is tempting to speculate that, after sperm-oocyte fusion, proteasomes penetrate the cytoplasm of the oocyte, associated tightly with the centrioles. Therefore, proteasomes can be considered as another paternal organelle (proteasomes can be considered *bona fide* organelles (Rubin & Finley, 1995)) contributed by the spermatozoon during fertilization. Our hypothesis is impossible to test directly in the human system for ethical reasons, however, it can be tested in the case of other mammalian species. It also raises many further questions.

The contribution of proteasomes from the single spermatozoon looks rather insignificant in the context that human oocytes contain huge amounts of proteasomes throughout their cytoplasm (Wojcik *et al.* 2000). However, mature human oocytes do not contain centrosomes, which must be contributed by the spermatozoon (Palermo *et al.*, 1994; Schatten, 1994; Van Blerkom & Davis, 1995; Sathananthan *et al.*, 1996; Hewitson *et al.*, 1997).

In the neck of the spermatozoon two centrioles are located, one proximal and one distal, which serve as a kind of basal body for the flagellum. These centrioles are a transformed centrosome, and proteasomes have been shown to be associated with the centrosomes in somatic cells. This association is very discrete in interphase cells (Wigley *et al.*, 1999), and becomes enhanced after the action of proteasome inhibitors (Wojcik *et al.*, 1996), while in mitotic cells it is much more evident (Amsterdam *et al.*, 1993; Wojcik *et al.*, 1995). It has been proposed that the centrosome can serve as an organiser of the ubiquitin-proteasome dependent proteolysis or a proteolysis centre, enriched in proteasomes and their activators (Wojcik *et al.*, 1996). An alternative hypothesis states that normally there is no enhanced proteolysis at the centrosome, but when misfolded proteins appear in the cell and/or proteasome function is impaired, they coalesce around the centrosome into an 'aggresome' (Johnston *et al.*, 1998).

It is possible, that the proteasomes contributed by the spermatozoa at fertilization somehow activate the pool of maternal proteasomes present in the oocytes by the appearance of the proteolysis centres. These can contribute to the proteolysis of various substrates including cyclins A and B, which take place after fertilization (Kubiak *et al.*, 1993) and it is well established that cyclins are degraded by the ubiquitin-proteasome pathway (Hershko, 1997).

Recently, it was reported that paternal mitochondria are tagged with ubiquitin during spermiogenesis, and that it appears to play a role in their later destruction in the early embryo (Sutovsky *et al.*, 1999). It is intriguing to speculate that the recruitment of the proteasomes to the necks of the spermatozoa may prevent their interaction with ubiquitinated mitochondria in the middle pieces. It is also possible, that the activation of maternal proteasomes by a paternal proteolytic centre is, therefore, related to the degradation of paternal mitochondria.

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MEETING ANNOUNCEMENT

Nordic Association for Andrology
Annual Meeting
Malmö, Sweden
31 August – 2 September 2000

The scientific programme will cover following topics*: seminal markers of male fertility, epididymal function, testicular biopsy, testicular paracrinology, male hormonal contraception, sex steroid receptors on spermatozoa, genetic variability in relation to regional variability in reproductive function, regional differences in semen quality*. Furthermore, leading researchers in the field of andrology will give their view on "*Andrology in the new millennium*".

The list of speakers includes: K-E Andersson (Lund), G Aumüller (Marburg), OPF Clausen (Oslo), T Cooper (Münster), G Forti (Florence), C Frimodt-Møller (Copenhagen), Gagnon (Montreal), B Jegou (Rennes), N Jørgensen (Copenhagen), H Lilja (Malmö), Å Lundwall (Malmö), S Michel (Rennes), O Söder (Stockholm), Van Steirteghem (Brussels), R Weber (Rotterdam), F Wu (Mancester), C-H Yeung (Münster).

If you would like to register and/or submit an abstract (oral or poster presentation) for the meeting, please contact:-

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