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Biochemical and Morphological Characterization of the Intra-Acrosomal Antigen SP-10 from Human Sperm[1]

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ABSTRACT

The human sperm protein SP-10 was previously defined as a "primary vaccine candidate" by a World Health Organization Taskforce on Contraceptive Vaccines. By one- and two-dimensional immunoblots, we show that SP-10, extracted from ejaculated human sperm, demonstrated a polymorphism of immunogenic peptides from 18 to 34 kDa, a pattern that was conserved from individual to individual and was not altered by reducing agents. The majority of the antigenic peptides possessed isoelectric points of approximately 4.9. Immunocytochemistry on testis sections indicated that SP-10 was localized to round spermatids and spermatozoa within the adluminal compartment of the seminiferous epithelium. Immunofluorescence showed that SP-10 was not associated with the surface of acrosome-intact, ejaculated sperm. Light and electron microscopic immunocytochemistry localized SP-10 throughout the acrosome, and electron microscopic evidence demonstrated a bilaminar array in association with the inner aspect of the outer acrosomal membrane and the outer aspect of the inner acrosomal membrane. After induction of the acrosome reaction with the ionophore A23187, SP-10 remained displayed on the sperm head in association with the inner acrosomal membrane and equatorial segment. The results indicate that the MHS-10 monoclonal antibody may be used as a marker of acrosome development in the human and as a probe to evaluate acrosome status. The results also support the hypothesis that inhibition of sperm-egg interaction by anti-SP-10 monoclonal antibody may occur as a result of antigen exposure following the acrosome reaction.

INTRODUCTION

It is generally assumed that plasma membrane antigens, as a class, provide the best potential immunogens for antifertility vaccine development (Anderson and Alexander, 1983). This generalization is based on the supposition that antibodies must interact with antigens accessible at the cell surface to cause cytolysis or immobilization. The human sperm protein, SP-10, is one of three molecules recently classified as a "primary vaccine candidate" by a World Health Organization Taskforce on Contraceptive Vaccines (Anderson et al., 1987). This designation was based upon several lines of evidence (Anderson et al., 1987) showing that a monoclonal antibody (MHS-10) to SP-10 (1) reacted with mature sperm as well as spermatogenic lineage cells; (2) lacked reactivity with somatic cells; and (3) inhibited sperm-egg interactions in the hamster egg penetration test (Rogers et al., 1979).

Our objective in the present study was to define several of the biochemical and morphological features of SP-10. The results show that SP-10 is an acidic, polymorphic protein, which is conserved in the human population. Arising during spermatogenesis within the nascent acrosomes of developing spermatids and localizing within the acrosome of intact sperm, SP-10 is not located on the plasmalemma but becomes exposed on the sperm surface after the acrosome reaction. SP-10 is thus a differentiation marker of acrosome development in humans and an example of an intra-acrosomal immunogen exposed prior to fertilization, offering a potential target for immunocontraception.

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MATERIALS AND METHODS

Hybridoma MHS-10 and Enzyme-Linked Immunosorbent Assay (ELISA) Selection

The antisperm monoclonal antibody MHS-10 was generated by conventional procedures (Galfre et al., 1977). Splenocytes from BALB/c female mice, immunized four times with 10^7 washed human sperm (suspended in incomplete Freund's adjuvant), were fused with the myeloma cell line SP2/0 (Shulman et al., 1978). The immunizing sperm were all obtained from blood type O donors. An ELISA (Engvall and Carlsson, 1976) was used to screen culture supernatants for antibody reactivity to sperm. Sperm (10^5) in a sodium carbonate bicarbonate (15 mM/34 mM) buffer, pH 9.2, were coated onto the wells of microtiter plates (Immulon II, Dynatech, Alexandria, VA) for 2 h at 37°C. Plates were blocked with a 0.01 M phosphate-buffered saline (PBS), 0.1% polyoxyethylene-sorbitan monolaurate (Tween-20) solution to decrease nonspecific binding. Peroxidase-labeled goat anti-mouse immunoglobulin G (IgG) (Hyclone Labs., Logan, UT), at a dilution of 1:1000 in PBS, 0.1% polysorbate, 1% bovine serum albumin (BSA), was used as a secondary antibody. The substrate 2, 2′-azino-bis-(3-ethylbenzthiazoline sulfonic acid; ABTS) was used to develop a colored reaction. Optical density (A_{405}) was read on a Microtiter Multiscan MC (Flow Laboratories, McLean, VA) after 30 min of incubation.

Hybridomas that elicited positive binding to sperm were expanded and cloned by limiting dilution. The immunoglobulin class of the monoclonal antibody was determined on culture supernatant using ELISA assay employing class-specific secondary antibodies (Hyclone Labs.) to mouse immunoglobulins. Ascites fluids were produced and assayed for immunoglobulin (Ig) concentration by an ELISA according to a published procedure (Herr et al., 1985).

Immunocytochemistry of Human Testis

Testes were obtained from elective orchiectomies for prostate carcinoma from patients untreated with steroids. Testes were fixed in 2% formaldehyde in 0.1 M phosphate buffer and embedded in paraffin. Ten-micrometer sections were mounted on gelatin-coated microscope slides, deparaffinized in a graded series of ethanols, and rehydrated in PBS. Sections were pretreated with 10% normal goat serum for 30 min, washed three times in PBS, and reacted with a 1:1000 dilution of monoclonal antibody MHS-10 or control IgG1 in 1% normal goat serum for 30 min. After washing, sections were treated with 1:100 dilution of goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) for 30 min, washed thrice, and incubated with mouse peroxidase-anti-peroxidase, 1:200, in PBS for 30 min, followed by three washes in PBS. Brown reaction product, indicating the location of the antigen, was developed with 0.05% diaminobenzidine with 0.015% hydrogen peroxide.

Immunofluorescence Microscopy

Motile sperm. Live ejaculated sperm were incubated 1.5 h in RPMI 1640 medium with 3.5% BSA at 37°C with 5% CO_2; 1.5×10^8 sperm were incubated for 1 h with MHS-10 antibody at 1:100 or control IgG1 at 1:100 diluted in RPMI. Samples were washed twice in medium and reacted with a 1:100 dilution of goat anti-mouse IgG-fluorescein isothyocyanate (FITC) (Jackson Immuno Research Laboratories) for 1 h. Samples were washed twice and observed as wet mounts. Fifty percent of sperm were motile at time of addition of primary antibody, 25% at addition of second antibody, and approximately 10% at time of scoring 1000 motile cells.

Effect of Triton X-100 or methanol permeabilization. A sample of 3×10^8 sperm was washed thrice in PBS containing 2 μM phenylmethylsulfonylfluoride. Sperm were fixed 30 min in 3% paraformaldehyde. Aliquots were permeabilized with 0.5% Triton X-100 or 100% methanol for 30 min at room temperature. Unpermeabilized samples were treated with PBS. After washing twice, samples were incubated with a 1:100 dilution of MHS-10 in PBS for 1 h at 37°C, followed by a 1:100 dilution of goat anti-mouse IgG. Preparations were washed twice and mounted in 90% glycerol, 0.25 M tris(hydroxymethyl)aminomethane (pH 7.5) and examined.

Routine method for scoring MHS-10 staining and acrosome-reacted sperm. On the basis of evidence (see Results) that membrane permeabilization exposes the SP-10 antigen, the following standard method was developed. Sperm from liquefied semen samples were washed twice in Ham's F-10 medium buffered with 0.1 M 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid (HEPES). For induction of the acrosome reaction, sperm suspensions were capacitated for 3 h at 37°C in Biggers, Whitten & Whittingham (BWW) medium

(Biggers et al., 1971) with 3.5% human serum albumin (HSA). Samples were acrosome-reacted for one-half h in 10 μM calcium ionophore A23187 in BWW containing 0.3% HSA. Sperm were cytocentrifuged onto a microscope slide, allowed to air-dry, and fixed with several drops of 3% paraformaldehyde for 45 min at room temperature. Slides were treated with 100% methanol for 20 min at room temperature and blocked with 10% normal goat serum (NGS) for 15 min. Slides were incubated with a 1:100 dilution of monoclonal antibody MHS-10 in 0.01 M PBS (pH 7.4), 1% NGS for 45 min at room temperature, then washed three times in PBS. A 1/100 dilution of FITC-conjugated goat anti-mouse IgG (Jackson Immuno Research) in PBS was used as a second antibody. Specimens were washed extensively and wet-mounted in 90% glycerol, 10% 0.1 M Tris (pH 7.5), with orthophenylene diamine added to prevent fading of fluorescence.

Electron Microscopic Immunocytochemistry

Testis tissue was fixed in 2% glutaraldehyde, 2% formaldehyde in 0.1 M cacodylate buffer, pH 7.3. A portion was post-fixed in 2% osmium tetroxide. Tissue was embedded in Araldite 502. Gold sections were cut on an ultramicrotome and then incubated with 0.2% ovalbumin for 30 min at room temperature to block nonspecific sites. Monoclonal antibody MHS-10 or control IgG1 was diluted 1:50 in 0.2% ovalbumin and reacted overnight with the sections at 4°C. After exhaustive washing in drops of PBS, sections were incubated for 2 h in a 1:25 dilution of Protein A gold (Janssen Life Sciences, Piscataway, NJ). Sections were then washed in PBS and stained for 10 min in 5% uranyl acetate and viewed in a JEOL 100CX electron microscope.

Western Blots

Donor sperm were washed in Ham's F-10 medium; frozen at −80°C in the presence of 5 mM benzamidine, 1 mM phenylmethylsulfonylfluoride, 2 μg/ml leupeptin, 2 μg/ml pepstatin; and thawed and extracted in 1% sodium dodecyl sulfate (SDS). One part extract was added to one part double-strength Laemmli buffer (Laemmli, 1970) in the presence or absence of β-mercaptoethanol. Proteins were analyzed by one- and two-dimensional electrophoresis according to the procedure of O'Farrell (1975). The method of Towbin et al. (1979) was used for electrotransfer. The nitrocellulose was blocked in 5% milk in PBS/0.5% Tween-20 and incubated in the MHS-10 monoclonal antibody (1/1000) in PBS/0.5% Tween-20, 1% milk overnight at 4°C; goat anti mouse IgG-peroxidase was used at a 1/5000 dilution. Control IgG1 monoclonal antibody was also diluted 1/1000. Silver-staining of protein spots on two-dimensional gels followed the procedure of Wray et al. (1981).

RESULTS

SP-10 is a Differentiation Antigen of Spermatogenesis

SP-10 was found to be expressed at a specific stage of sperm differentiation in the human testis. Immunohistochemical examination of paraffin-embedded testes (n = 3) exposed to the MHS-10 monoclonal antibody (isotype: IgG1) revealed binding to adluminal spermatids and mature sperm within the seminiferous tubules (Fig. 1A,B). Control sections of human testis incubated with another IgG1 monoclonal antibody (Fig. 1C) showed no immunoreaction product. Within round spermatids, immunostaining was frequently observed in crescent-shaped structures as well as in smaller ovoid granules (Fig. 1B, arrowheads). Groups of similarly stained spermatids that demonstrated either crescent-shaped or granular immunoreaction patterns (as in Fig. 1B) were observed in cross-sections of single seminiferous tubules. This finding was consistent with previous observations in the human testis that germ cells in several stages of differentiation may coexist in any cross-section of a seminiferous tubule (Clermont, 1963; Schulze and Rehder, 1984). Not all regions of the seminiferous epithelium demonstrated staining, suggesting either a lack of expression of SP-10 in some stages of spermatogenesis or possible detachment of some cells from the seminiferous epithelium in the paraffin-embedded material. Further study of the immunocytochemical staining patterns in plastic sections of testis at the light and electron microscopic levels are underway to define these possibilities. Basal spermatogonia, Sertoli cells, spermatocytes, and cells within the testicular interstitium showed no immunoreactivity.

SP-10 Resides within the Acrosome of Intact Sperm

Immunofluorescence microscopy showed SP-10 localized to the head of human sperm. Motile, nonper-

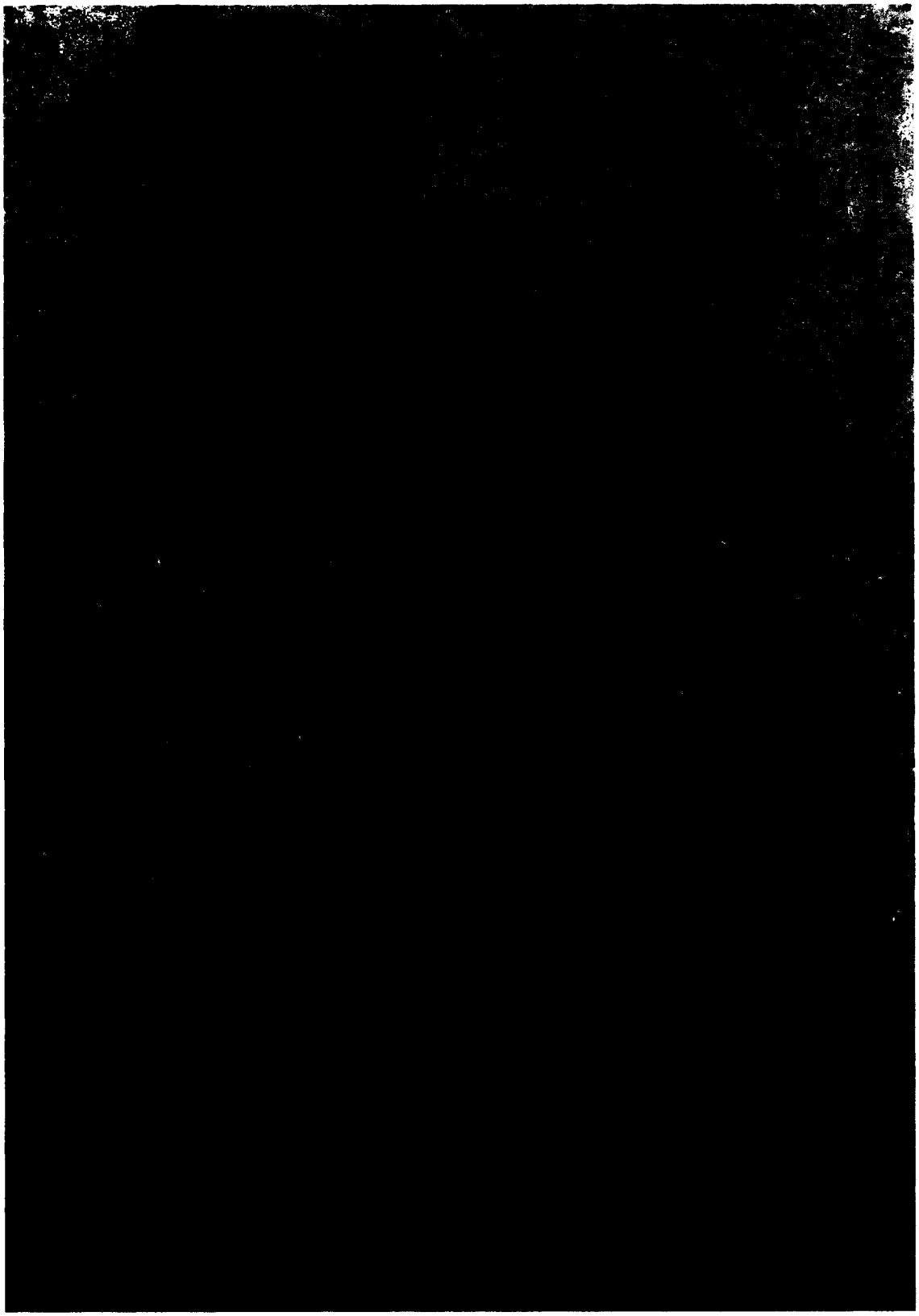

FIG. 1. Immunohistochemical localization of SP-10 within seminiferous tubules of the human testis. *(A)* Cross-sections of seminiferous tubules reacted with the MHS-10 monoclonal antibody (1:1000) demonstrate dark reaction product in the adluminal compartment (×180). *(B)* At higher magnification, both crescent-shaped and smaller granular reaction products *(arrowheads)* are observed in cohorts of similar stage germ cells within a single seminiferous tubule (×720). *(C)* Tissue section treated with the control mouse IgG1 shows no staining (× 180).

meabilized sperm (n = 1000) that were incubated with the MHS-10 monoclonal antibody and reacted with a fluorescent secondary anti-mouse antibody showed no immunofluorescent staining of the sperm (data not shown). This indicated that SP-10 was not present on the surface plasma membrane of intact sperm at detectable levels. Sperm that were air-dried on slides, fixed with 3% paraformaldehyde, permeabilized with 0.5% Triton X-100 or methanol, and then reacted with the monoclonal antibody and a fluorescent secondary anti-mouse antibody stained in a cap-shaped fluorescent pattern. This pattern, similar to the known morphology of the acrosome, occurred in >90% of sperm in each sample (Fig. 2A). These results indicated that membrane-permeabilizing treatments rendered SP-10 accessible to antibody binding.

Ultrastructural Localization Indicated That SP-10 Is Associated with the Acrosomal Membranes

Fine-structural studies were performed to localize SP-10 at higher resolution. Mature, ejaculated spermatozoa were fixed in 2% paraformaldehyde and 2% glutaraldehyde, prepared for electron microscopy, and immunolabeled on plastic sections with the MHS-10 monoclonal antibody and 10 nm gold particles coated with Protein-A. A concentration of gold particles was observed over the acrosomal compartment (Fig. 3). In sections where a portion of the acrosome was sectioned obliquely (as in Fig. 3 at the sperm apex), gold particles were observed in a bilaminar array. This suggested that in mature, intact sperm, SP-10 is nonuniformly distributed within the acrosome and is associated with the inner and outer acrosomal membranes. Precise assignment of antigen location at the fine-structural level was difficult in these preparations, because post-fixation in osmium tetroxide, which defines cellular membranes, was found to destroy antigenicity. However, by comparing nonosmicated, immunolabeled specimens to osmicated sperm, the position of the acrosomal membranes was determined to correspond to the electron-lucent regions indicated at the arrowheads in Figure 3. This suggested that SP-10 is located on the faces of both inner and outer acrosomal membranes adjacent to the acrosomal matrix in mature, intact, ejaculated sperm.

Biochemical Characterization

The molecular characteristics of SP-10 were studied by Western blots of one- and two-dimensional gels on which sperm homogenates were electrophoresed. The pattern of immunoreactive sperm proteins observed on Western blots of a 10% acrylamide, one-dimensional SDS-polyacrylamide electrophoretic gel allowed resolution of at least 14 distinct peptide bands (Fig. 4B), which ranged from 18 to 34 kDa. Sperm homogenates treated with SDS and the disulfide bond-reducing agent, β-mercaptoethanol, were compared to homogenates that were not exposed to the reducing agent (Fig. 4B). The pattern of immunoreactive peptides was identical whether or not β-mercaptoethanol was present, indicating that reduction of disulfide bonds did not alter the apparent molecular masses of the immunoreactive peptides.

Figure 4B shows that immunoreactive SP-10 from different individuals was very similar. The relative intensity of antibody reactivity with any one peptide band was similar in different individuals, as was the presence in each sperm homogenate of the full complement of 14 distinct immunoreactive peptide bands (Fig. 4B). To date, no sperm sample tested, by either immunofluorescence or Western blots (n = 60), has failed to react with the MHS-10 monoclonal antibody, indicating that SP-10 is highly conserved in the human population.

Silver stain of a sperm homogenate that was electrophoresed on a two-dimensional gel showed many protein spots possessing isoelectric points over the pH range 4.3 to 6.5 (Fig. 5A). The MHS-10 monoclonal antibody immunoreacted (Fig. 5B) with a series of peptide spots that ranged in apparent molecular mass from 18 to 34 kDa. Immunoreactive peptides with apparent molecular masses from 24 to 34 kDa had isoelectric points of approximately 4.9, whereas the immunoreactive peptides in the 18 kDa range were slightly more basic with pIs from 5.1 to 5.4.

SP-10 Remains Associated with the Sperm Head after the Acrosome Reaction

It is well known that certain constituents of the acrosomal matrix diffuse from the acrosome during the acrosome reaction, when the outer acrosomal membrane fuses with the sperm plasma membrane (Nagae et al., 1986; Yudin et al., 1988). Figure 2B shows immunofluorescent staining patterns obtained when the MHS-10 monoclonal antibody was reacted with sperm samples treated with the calcium ionophore A23187 (which induces some of the sperm to undergo the acrosome reaction). Ionophore-treated populations contained increased numbers of sperm showing equatorial bars (Fig. 2B, thin arrowheads) as well as sperm dis-

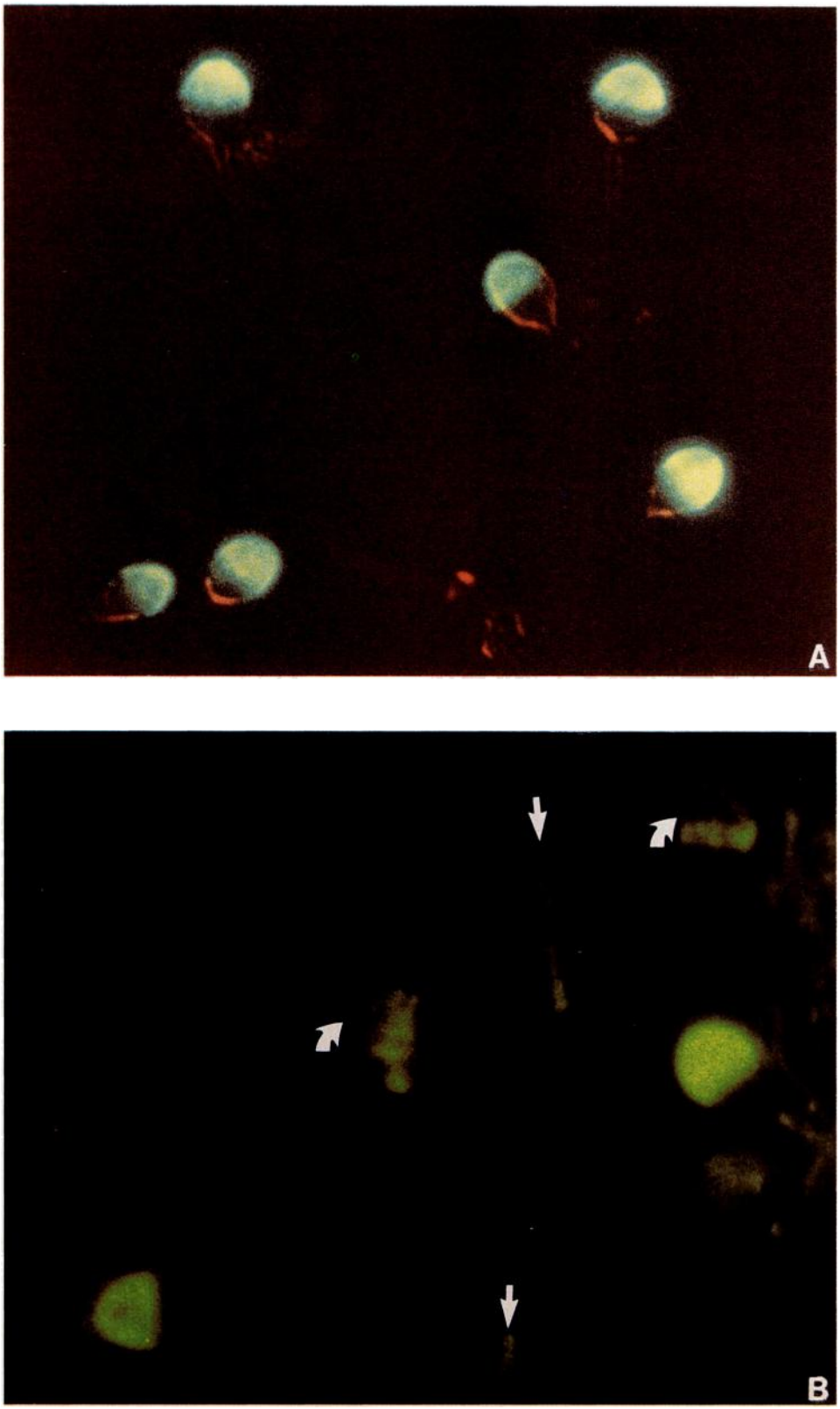

FIG. 2. Immunofluorescent light micrographs localizing SP-10 in ejaculated human sperm. *(A)* A combination phase-contrast and fluorescent image demonstrates cap-shaped fluorescence over the anterior portion of the sperm head (× 2870). *(B)* Sperm after artificial induction of the acrosome reaction with the calcium ionophore A23187. In the experiment from which the above photo was taken, 47.5% of sperm showed fully fluorescent caps, 20.3% showed faint fluorescent caps *(curved arrow)*, 22.4% showed equatorial bars *(straight arrow)*, and 9.9% of the sperm were unstained (×3350).

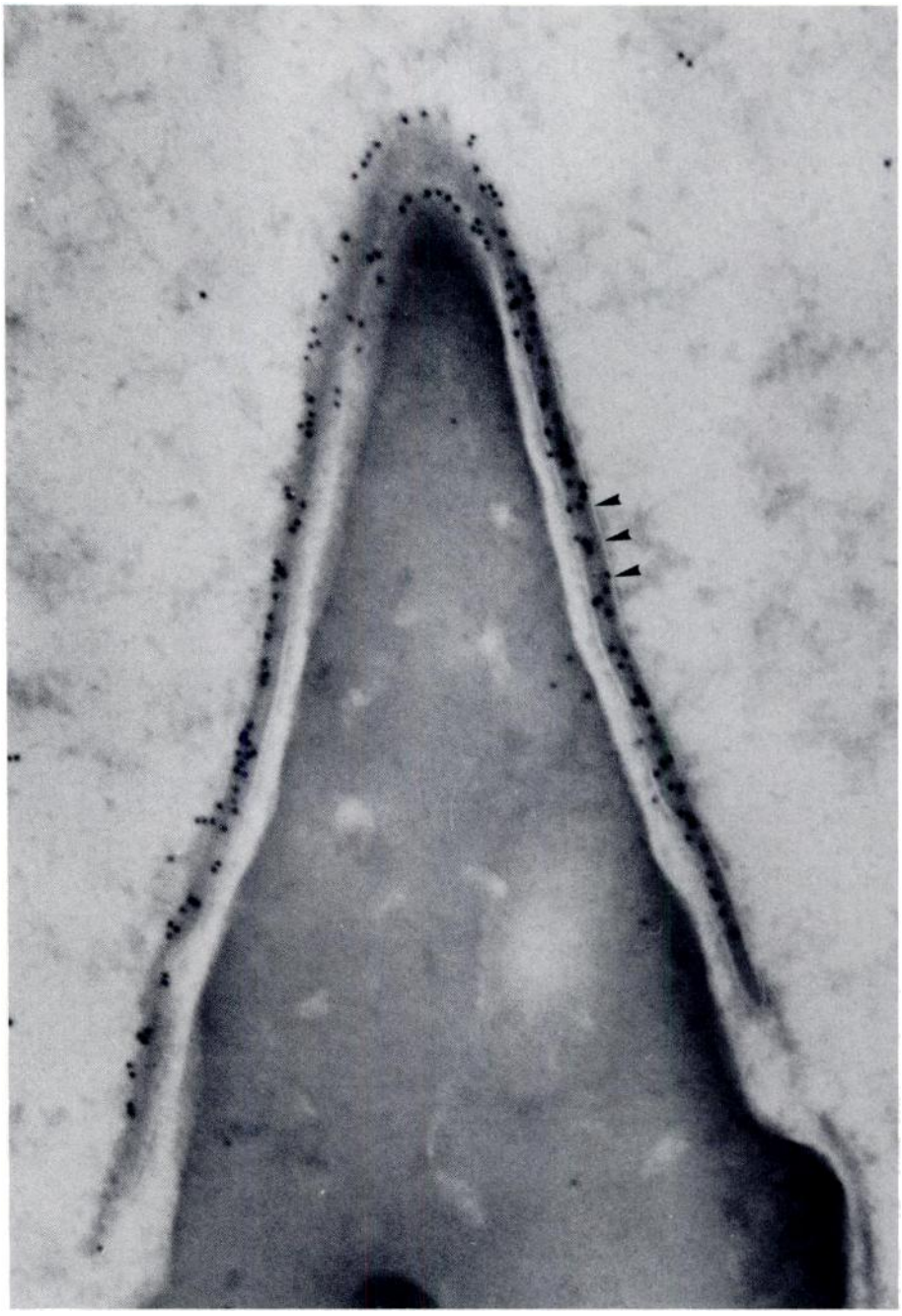

FIG. 3. Electron micrograph of human sperm head fter reaction with monoclonal antibody MHS-10 and Protein-A gold. Gold particles are observed over the acrosomal compartment. In regions where the acrosome was sectioned obliquely, as at the sperm apex, the gold particles follow a bilaminar distribution. *Arrowheads* indicate location of acrosomal membranes, which are electron-lucent in this unosmicated material (×98,300).

playing either faint caps or faint caps and equatorial bars together (Fig. 2B, thick arrowheads). These light microscopic results indicated that SP-10 remains, in part, associated with the sperm head after the acrosome reaction. The faint caps suggested that SP-10 persists on the inner acrosomal membrane, which is exposed on the sperm head after the acrosome reaction (Nagae et al., 1986; Yudin et al., 1988), and the fluorescent equatorial bars indicated retention of SP-10 in association with the sperm's equatorial segment. Further fine-structural studies are underway to extend these observations.

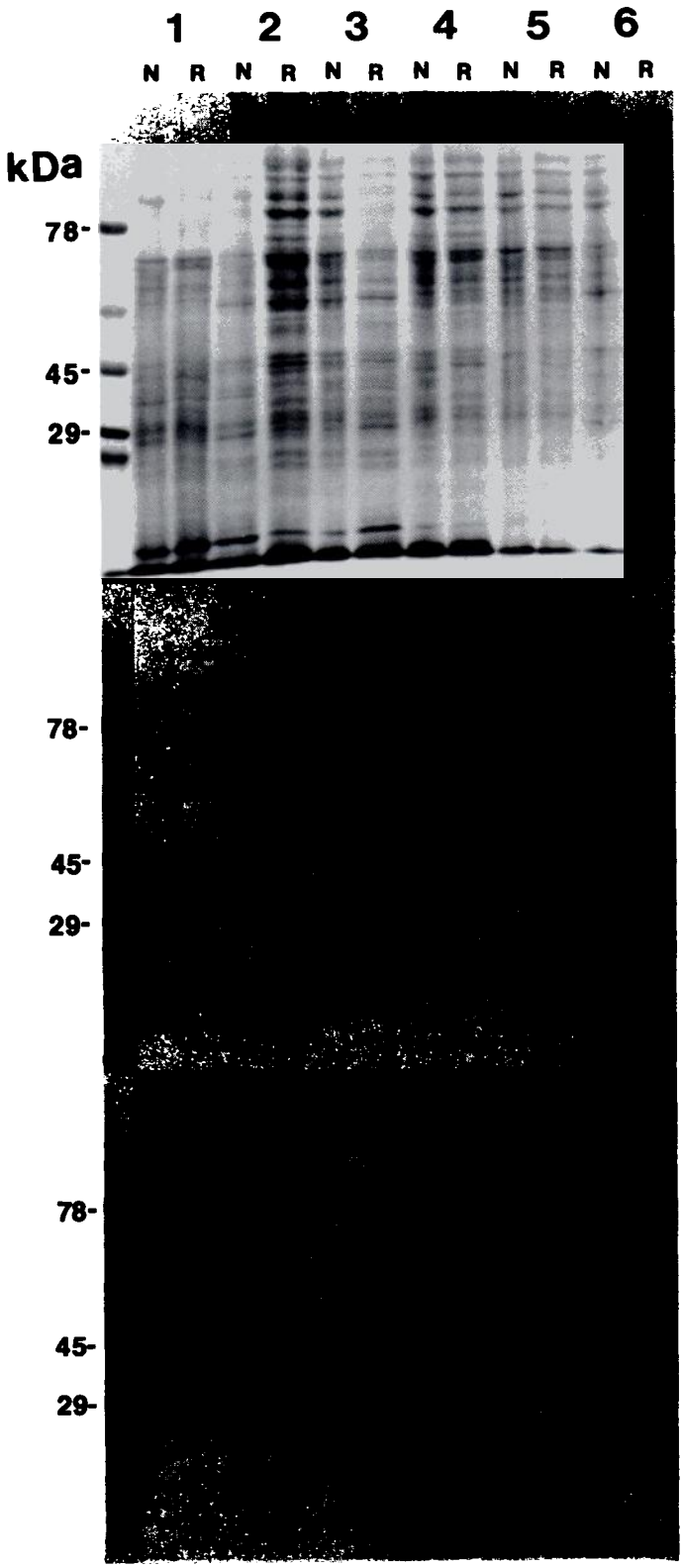

FIG. 4. One-dimensional SDS-polyacrylamide electrophoretic nitrocellulose electroblot stained with amido black *(A)* and identical nitrocellulose sheet reacted with the MHS-10 monoclonal antibody *(B)*; control IgG1 *(C)*. Sperm extracts from 6 donors (1 – 6) contained β-mercaptoethanol (lanes marked *R* = reduced) or lacked this agent (nonreduced = N). Twenty-five micrograms of protein was run per *lane*. The pattern of SP-10 immunoreactive peptides is identical both between persons and in reduced and nonreduced extracts.

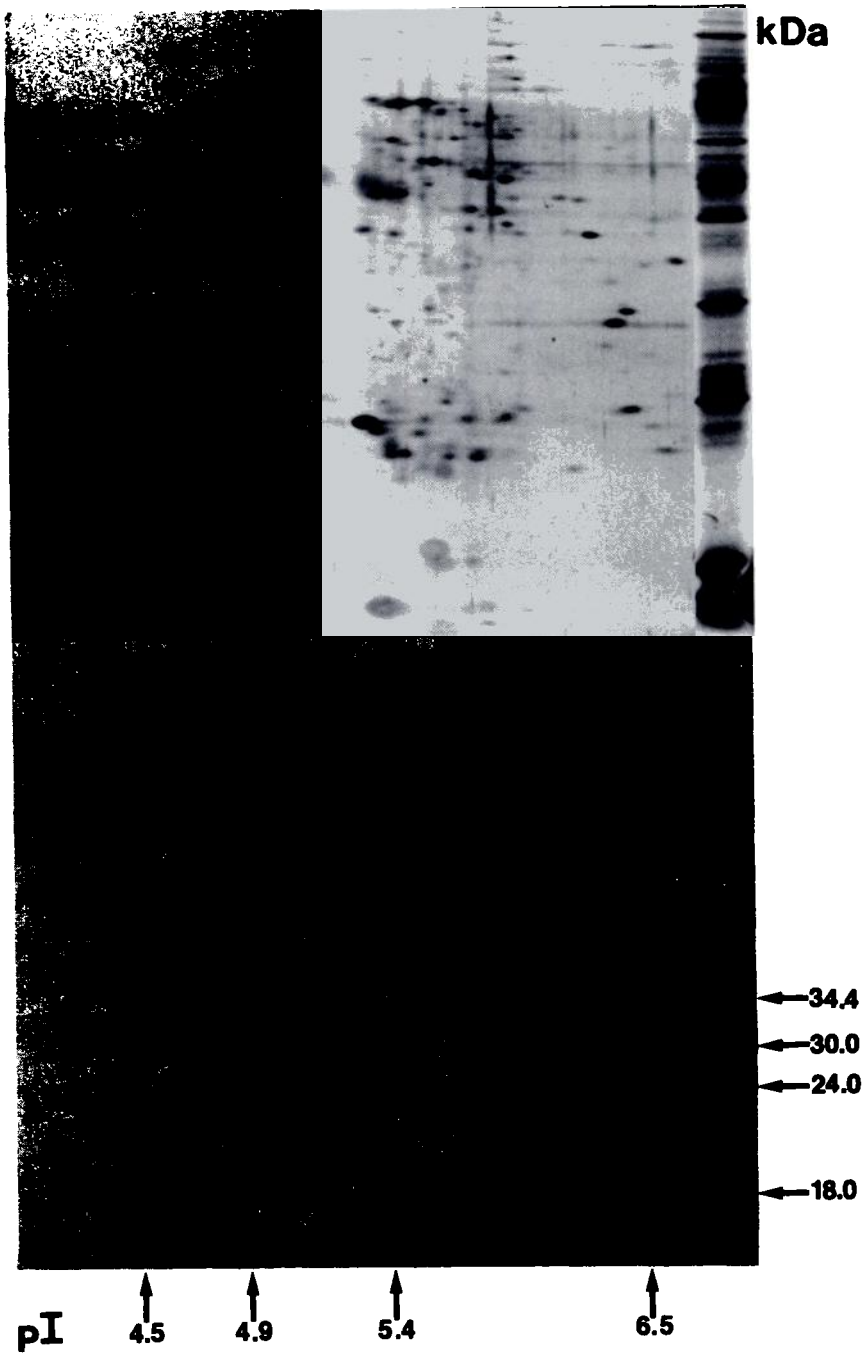

FIG. 5. Silver-stained two-dimensional gel *(A)* and immunoblot *(B)* using MHS-10 monoclonal antibody on proteins extracted from human sperm. A one-dimensional *lane* showing the silver stain and immunoblot pattern of the sperm extract lies to the *right* of each *figure*. Molecular mass in kDa and isoelectric points *(pI)* are indicated on the *right* and *bottom margins*, respectively. *Arrows* on the silver stain above indicate the location of SP-10 proteins *(AP)* at 34 and 30 kDa, which may be compared to bands and spots of similar mass on the immunoblot below. Two-dimensional and one-dimensional gels were loaded with 75 and 15 μg of sperm protein, respectively. Immunoreactive SP-10 peptides from 24 to 34 kDa have a pI of 4.9; the 18 kDa spots range in pI from 5.1 to 5.4.

DISCUSSION

The observations that the MHS-10 monoclonal antibody reacts only with round spermatids and subsequent stages of spermiogenesis on testis sections and localizes within the acrosome at the electron microscopic level, together with the report that somatic tissues are nonreactive with the MHS-10 monoclonal antibody (Ander-

son et al., 1987), indicate that SP-10 may be classified as a "differentiation antigen" (Bennett et al., 1972), i.e., a tissue-specific molecule expressed at a precise stage of human spermatogenesis. MHS-10 immunoreaction product was evident in the seminiferous epithelium as small ovoid granules adjacent to the nucleus of round spermatids. This staining, indicative of the earliest stage of spermatogenesis at which SP-10 was detectable, probably corresponds to the nascent acrosomal vesicle and/or peri-nuclear Golgi region. The MHS-10 monoclonal antibody thus may offer a useful marker of acrosome development in humans. One clinical application of this antibody probe may be in the diagnosis of the incidence of immature germ cells (Golgi-phase spermatids and subsequent steps) in semen samples with so-called "round cell syndrome" (Belsey et al., 1980; Jassim and Festenstein, 1987).

The absence of cross-reactivity in somatic tissues coupled with its stage-specific expression during germ cell differentiation is also germane to the possible utility of SP-10 as a contraceptive vaccine immunogen. Potential problems of autoimmunity, which would be anticipated if common somatic antigens were used as vaccine immunogens, may not be found with SP-10.

Our immunofluorescence evidence indicated that in acrosome-intact, membrane-permeabilized sperm, SP-10 localized in a cap-shaped immunofluorescent pattern that appeared to encompass the entire acrosome in 90% or more sperm from a given donor. There was no evidence that the MHS-10 antibody recognized its cognate antigen on the plasmalemma of living sperm. The report of the WHO workshop (Anderson et al., 1987, p. 249) concluded that the MHS-10 antibody (S20) showed "reactivity. . .with abundant surface antigens on mature sperm." We wish to emphasize that our results do not agree with this conclusion insofar as it applies to acrosome-intact sperm.

Our results show that after ionophore-induced acrosome reaction, an increase was noted in the number of sperm displaying fluorescent bars or fluorescent bars together with fainter fluorescent caps. We interpret the reduced immunofluorescence of the cap (faint cap) to indicate that after the acrosome reaction SP-10 is displayed on the sperm surface, most likely in association with the inner acrosomal membrane. The retention of immunofluorescence after the acrosome reaction in a belt-like bar probably represents retention of SP-10 within the equatorial segment. In many sperm, the equatorial bar immunofluorescence, although covering a much smaller region than the fluorescent cap, appeared to be of the same intensity as the complete cap pattern, indicating that the amount of SP-10 within the equatorial segment is similar before and after the acrosome reaction. The immunofluorescence data is not of sufficient resolution to determine whether SP-10 remains localized to the inner and/or outer acrosomal membranes and matrix of the equatorial segment, or possibly to all of these subdomains after the acrosome reaction, or whether SP-10 is redistributed to include the plasma membrane overlying the equatorial segment. Electron microscopic studies are in progress to answer this question.

The WHO-sponsored multicenter study presented evidence that the MHS-10 monoclonal antibody (S20) inhibited sperm-egg interactions in the hamster egg penetration test (Anderson et al., 1987). Our model to explain this result postulates that the SP-10 antigen, although sequestered within the limits of the acrosomal membranes in intact, non-acrosome-reacted sperm, is accessible to the actions of the MHS-10 antibody after the acrosome reaction. Current concepts regarding key events of fertilization are relevant to this model. During fertilization, human sperm must undergo the acrosome reaction in order to penetrate the zona pellucida (Singer et al., 1985) and fuse with the egg plasma membrane (Sathananthan and Chen, 1986). After the acrosome reaction, the acrosomal contents are externalized and the inner acrosomal membrane becomes the limiting membrane of the anterior sperm head (Nagae et al., 1986; Yudin et al., 1988). Most sperm observed on the human zona after one minute of binding in vitro have intact acrosomes (Cross et al., 1988). The numbers of acrosome-reacted sperm on the zona then rapidly increase with time, because the zona acts as a potent inducer of the acrosome reaction (Cross et al., 1988). After penetration of the zona, the site of initiation of fusion between sperm and egg in humans, as in other eutherian animals, is thought to occur between the plasma membrane over the equatorial segment and the egg plasma membrane (Bedford et al., 1979; Sathananthan et al., 1986). Important questions remain to be elucidated regarding immunocontraception based on SP-10 or other intra-acrosomal immunogens. Are the sperm of humans or other primates acrosome-reacted prior to or during zona binding during in vivo fertilization in the oviduct? Can antibodies in oviductal fluids be induced by vaccination to reach sufficient levels to agglutinate, immobilize, or lyse acrosome-reacted sperm? Can such antibodies in secretions of the female tract gain access in vivo to antigens on the inner

acrosomal membrane or equatorial segment to block either sperm penetration of the zona or fusion with the egg membrane? Some of these questions will be addressed when trials of an SP-10-based vaccine are undertaken in primates.

A common assumption regarding selection of appropriate sperm immunogens for contraceptive vaccine development is that the target molecules should be surface components accessible to humoral or cellular immune effectors (Anderson and Alexander, 1983). Although the intra-acrosomal localization of the SP-10 peptides in mature, non-acrosome-reacted sperm appears at first glance not to fulfill this caveat, the remodeling of the sperm head membranes that accompanies the acrosome reaction opens the possibility that, as a class, constituents of the acrosome, although sequestered from the immune system in intact sperm, should not be dismissed as candidates for contraceptive vaccines without examination of their fate following the acrosome reaction.

Mitigating this notion are data on immunization with acrosin and hyaluronidase. Like SP-10, acrosin is retained on the inner acrosomal membrane and equatorial segment after the acrosome reaction (Tesařík et al., 1988). In rabbits, antibodies formed in response to systematic immunization with acrosin were found only in low amounts in oviductal fluid (Syner et al., 1979). Immunization with hyaluronidase also has not resulted in significant reductions in fertility (Morton and McAnulty, 1979). On the other hand, studies with guinea pig sperm have provided remarkable evidence that full but reversible contraception can be achieved by immunizing female animals with the purified sperm protein, PH-20 (Primakoff et al., 1988b). This 64 kDa molecule is present on both the plasma membrane and, following the acrosome reaction, the inner acrosomal membrane (Primakoff et al., 1985; 1988a; Myles and Primakoff, 1984; Cowan et al., 1986). PH-20 may play a role in sperm binding to the zona pellucida (Primakoff et al., 1985) and appears to undergo proteolysis during the acrosome reaction (Primakoff et al., 1988a). Although SP-10 and PH-20 appear to be different molecules on the basis of apparent molecular mass and immunoreactivity, they share the property of persistence on the sperm head following the acrosome reaction. The remarkable effectiveness of PH-20 in eliciting a contraceptive effect in guinea pigs (Primakoff et al., 1988b), suggests that certain antigens associated with acrosome-reacted sperm may be effective targets for immunocontraception. SP-10 appears to be a model molecule in humans for this type of intra-acrosomal immunogen. We are currently examining the sera of infertile men and women with high titers of anti-sperm antibodies for specific recognition of SP-10.

A number of methods including monoclonal antibody and lectin probes as well as multiple dye techniques have been used to score the acrosome reaction (Wolf et al., 1985; Cross et al., 1986; Lee et al., 1987; Berger et al., 1989). Because the MHS-10 monoclonal antibody is directed to an intra-acrosomal antigen, which changes from a cap-shaped immunofluorescence pattern to a faint cap and/or bar during the acrosome reaction, it may also be useful clinically in assessing acrosomal status.

The changes following the acrosome reaction in immunofluorescence observed with SP-10 are similar to patterns seen with *Pisum sativum* agglutinin (Cross et al., 1986) and the T5 and T6 monoclonal antibodies (Ochs et al., 1986). The latter probes recognize a keratin-like protein with a pI of 6.9 that is also present in skin. The pI of 4.9 observed for SP-10 make it unlikely that T5 and T6 recognize the same antigen as MHS-10. The 1A.1 monoclonal antibody, which yields a fluorescent cap pattern on human sperm (Villarroya and Scholler, 1986), localizes to the plasma membrane domain (Villarroya and Scholler, 1987), indicating dissimilarity of its cognate antigen to SP-10.

We observed a high degree of similarity between individuals in the immunoreactive forms of SP-10 on Western blots, as well as consistent immunofluorescent localizations on each individual's sperm, indicating that SP-10 is conserved in the human population. This knowledge is essential in choosing a contraceptive vaccine molecule, because it must be present on most, if not all, sperm for a vaccine to achieve the widest possible effectiveness. The multiple forms of SP-10 peptides that are identified by Western blotting may represent post-translational modifications, proteolytic processing of the protein within the acrosome, multiple gene products, or several of these possibilities acting in concert. The high degree of similarity between individuals on Western blots suggests that, whichever of these alternatives is acting to produce the polymorphism in antigenic peptides, the mechanisms are operating similarly in different individuals. The fact that reduction did not alter the pattern of immunoreactive SP-10 peptides suggests a lack of interchain and few or no intrachain disulfide bonds in SP-10. The MHS-10 monoclonal antibody has been used successfully to isolate cDNA clones from a lambda gt11 library of human testis,

suggesting that the antibody recognizes a proteinaceous rather than carbohydrate epitope (data not shown). Structural information on the amino acid sequence of SP-10 peptides will be developed from the sequence of encoding cDNAs, a study currently underway.

Electron microscopic localizations in intact, ejaculated human sperm indicate that SP-10 is asymmetrically disposed within the acrosomal matrix, associating in many sperm with the faces of both inner and outer acrosomal membranes adjacent to the acrosomal matrix. Because the polymorphism of SP-10 is not completely understood at the level of amino acid sequence and a function for the SP-10 polypeptides has not yet been determined – aside from their potential as vaccine immunogens, an understanding of the significance of the apparent asymmetry of SP-10 in the acrosome can only be discussed in a general sense. Knowledge of the spatial organization of various molecules within the acrosomal matrix and acrosomal membranes in intact and acrosome-reacted sperm is currently in its infancy (Huang and Yanagimachi, 1985). The inner acrosomal membrane may function (1) as a scaffold for zona lysins, which may be retained on the exposed inner acrosomal membrane and equatorial segment after the acrosome reaction (Tesařík et al., 1988); (2) as a rigid structure that maintains integrity during zona penetration; and (3) in early recognition/adhesion with the oolemma (Huang and Yanagimachi, 1985; Singer et al., 1985). In guinea pig sperm, Gerton and coworkers (1988) recently presented immunofluorescent evidence that an antigen recognized by monoclonal antibody 1D4 appeared to be restricted to the boundaries of the acrosome. Cytochemical observation (Talbot and DiCarlantonio, 1985) as well as enzymatic studies (DiCarlantonio and Talbot, 1988) of guinea pig sperm support the concept that acrosomal contents are compartmentalized within the acrosomal vesicle and are sequentially deployed during the acrosome reaction. This is supported by evidence in ram sperm that subcompartments in the acrosome appear to vary in their solubility (Shams-Borham and Harrison, 1981). In guinea pig sperm, electron microscopy has shown that the acrosomal matrix consists of distinct zones of electron density (Fawcett and Hollenberg, 1963). An electron-dense layer of filaments (acrosomal lamina) associated with the luminal surface of the outer acrosomal membrane has been noted in this species (Olson et al., 1988). A suggestion has been made that this layer represents a cytoskeletal-like complex that may direct the type of membrane-fusion events occurring during the acrosome reaction (Flaherty and Olson, 1988). The bilaminar electron microscopic localizations of SP-10 associated with the inner and outer acrosomal membranes makes homology with the guinea pig acrosomal lamina an attractive hypothesis. It is tempting to speculate that the asymmetrical distribution of SP-10 indicates the molecule contains a hydrophobic domain which directly inserts into the acrosomal membranes.

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