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Characterization of Monoclonal Antibodies Against Human Sperm Antigens by Immunoassays Including Sperm Function Assays and Epitope Evaluation

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ABSTRACT: **Fifteen monoclonal antibodies (MAbs) raised against human sperm cells were evaluated for reactions against human sperm by indirect immunofluorescence, immunocytochemistry, agglutination, complement-dependent immobilization, cervical mucus penetration, and hamster egg penetration assays. The MAbs were analyzed for specificity by immunofluorescent reactions with peripheral blood lymphocytes and sperm and classified into three main groups based on regional staining, ie, acrosome, plasma membrane, or tail. One MAb (218) bound to the sperm neck. Three MAbs, (80, 85, and HS-126) were found to react with lymphocytes. Three of five acrosome-reactive MAbs (11, 63, 106), two of five tail-staining MAbs (97, HS-30), and the neck reactor (218) showed significant to highly significant inhibition of sperm penetration of eggs but without significant effects on sperm agglutination, immobilization, or the mucus penetration assay. The three nonspecific MAbs gave strong plasma membrane reactions in the agglutination and immobilization assays and also caused highly significant inhibition of sperm penetration of both cervical mucus and zona-free ova. Preliminary analysis of the complementary antigens suggested that epitopes reacting with MAbs 33 (acrosome) and 85 (plasma membrane) were carbohydrate chains on glycoproteins. Three of five MAbs recognizing tail antigen, the neck-staining MAb, and the nonspecific MAb (HS-126) appeared to be reactive against glycolipid moieties. Seven of the 12 specific MAbs also reacted in indirect immunofluorescence with mouse and rabbit sperm in patterns similar to those observed with human sperm. (Am J Reprod Immunol Microbiol. 1987; 13:108–114.)**

Key words: Sperm monoclonal antibodies, antibody inhibition of sperm function, sperm antigens, sperm-antibody reactions.

INTRODUCTION

Immunologic reactions against sperm antigens have become recognized as contributing factors to infertility in men and women.[1] Studies have indicated that multiple sperm antigens are involved in the auto- and isoimmune humoral responses associated with infertility.[2,3] The humoral response may be restricted to antibodies circulating systemically, to antibodies secreted locally in the reproductive tract, or to a combination of the two systems. Antisperm antibodies can exert inhibitory effects on sperm cell function by affecting survival in the female reproductive tract, penetration through cervical mucus, and interactions with the ovum and also may interfere with early embryo survival in animals and women.[1,4]

Since studies suggest the involvement of multiple sperm antigens with human immunologic infertility, the identification, characterization, and isolation of the relevant antigens will aid in developing better diagnostic procedures and therapies for immunologic infertility as well as being important for the field of immunologic contraception. One approach to examine antigen constituency of sperm cells is the use of hybridoma technology and the production of monoclonal antibodies. Specific antigens of sperm have been identified, isolated, and functionally characterized by use of monoclonal antibody methodology.[5–7] We have generated numerous monoclonal antibodies against human sperm, some of which have been previously described.[8,9] In the present study a group of 15 monoclonal antibodies (MAbs) raised against human sperm from a large group of new clones was selected on the basis of generally strong reactions in indirect immunofluorescence representing different regional binding on the sperm and in agglutination and immobilization reactions with sperm. These clones were further characterized by effects on two sperm function assays, and finally, the nature of the complementary antigens was characterized in preliminary chemical and enzyme studies.

MATERIALS AND METHODS

Monoclonal Antibody Production

Monoclonal antibodies that were selected for this study were secreted by hybridomas generated through the cell fusion between NS-1 myeloma cells and spleen cells of male BALB/c mice that had been immunized with human sperm antigens. Detailed procedures for immunization, cell fusion, and cloning of hybrid cells were similar to those reported previously.[9] Human spermatozoa that had been washed three times with 0.5 M KCl in phosphate-buffered saline (PBS) were used as immunogens for immunizations of BALB/c mice. Following cell fusion and initial clonings in a semisolid medium containing methylcellulose, the hybrid cells were then subcultured in RPMI 1640 medium containing 10% fetal calf serum and HT (hypoxanthine and thymidine). The culture supernatants were screened for the secretion of sperm antigen-specific monoclonal antibodies by indirect immunofluorescent assay using live human spermatozoa for staining. Out of 1,500 hybrid clones that were initially picked, about 200 showed binding to live human sperm by this assay procedure.

Immunoglobulin Subclasses of Monoclonal Antibodies

Immunoglobulin subclasses of monoclonal antibodies were determined by the double immunodiffusion-in-gel

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method. Briefly, the culture supernatant containing monoclonal antibody was first concentrated tenfold by 40% ammonium sulfate fractionations. Rabbit anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, and IgM (from Bionetics, Charleston, SC) were employed for double immunodiffusion experiments with the concentrated culture supernatant.

Monoclonal Antibody Fragmentation

Fab fragments were prepared from ammonium sulfate precipitates of ascites by papain digestion following the procedures outlined by Goding.[10]

Indirect Immunofluorescence Assay

Spermatozoa were obtained from the ejaculates of volunteer healthy men, pooled ejaculates of male New Zealand white rabbits collected with an artificial vagina, and the cauda epididymides of male CD-1 mice. The sperm cells were washed twice by centrifugation at 500 g for 10 min with $\geqslant$10 volumes of PBS pH 7.5, the sperm pellet after the second wash was divided, and one portion was resuspended in PBS and the other portion in 0.5 M KCl in 0.05 M Tris-HCl buffer (pH 7.5). The divided samples were allowed to stand with frequent resuspension by inversion of the tubes for 10 min at room temperature. The samples were centrifuged, washed an additional time with PBS, resuspended in PBS, and air-dried smears were prepared for each sample. The slides of sperm exposed only to PBS were then fixed in absolute methanol for 30 min at room temperature, dried, and stored along with the KCl-prepared sperm slides at 5°C in a dessicator until used. Undiluted hybridoma cell supernatants or diluted ($\geqslant$1:10) ascites were used as primary antibody, and affinity-purified sheep anti-mouse IgG (H & L chain) fluorescein-conjugated immunoglobulin (Cappell Labs) diluted 1:100 in PBS was the second antibody as described earlier.[9] Controls for the primary antibodies included PBS, hybridoma cell culture medium, and ascites induced by NS-1 myeloma cells. Before application of MAbs, the sperm smears were rehydrated with 5% sheep normal serum in PBS for 10 min.

Immunofluorescent staining of live human sperm was performed as follows: 10 μl of freshly ejaculated sperm (4×10^5) once washed in PBS and resuspended in PBS containing 5% sheep normal serum was mixed and incubated with 25 μl of hybridoma cell supernatant or ascites ($\geqslant$1:10 dilution) in a 1.5-ml microcentrifuge tube for 45 min at room temperature; 1 ml of PBS was added per tube, and the samples were centrifuged at 500g for 6 min. The sperm pellets were washed a second time, resuspended in 25 μl of PBS and air-dried on microslides for staining with labeled second antibody and reading as described above. Sperm motility after the incubation and first wash was determined and was found to be $\geqslant$55% in all samples. Each of the immunofluorescent assays was run on sperm cells of at least three different donors.

Fluoresceinated Concanavalin A (Sigma Chemical Co.) at 10 μg/ml of PBS containing 1% bovine serum albumin was used to fluorescently label sperm cells. Sperm preparations were exposed to the labeled lectin for 10 min before washing twice with PBS, coverslipping, and observing.

Agglutination and Immobilization Activities

These antibody activities were analyzed, respectively, by modified assays of Friberg[11] and Isojima.[12] Ascites fluid was clarified by centrifugation (800g, 10 min) and heated at 56°C for 30 min before use in the assays.

Sperm Cell Treatments

Suspensions of KCl sperm as well as the dried sperm samples were subjected to the following different treatments: 20 mM periodate, 0.5% NP·40 detergent, 0.2 M lithium diiodosalicylate (LIS), 0.1% trypsin, 0.1% chymotrypsin, 0.1% pronase, and 0.1 u/ml neuraminidase (type X). Periodate and neuraminidase were prepared in 50 mM sodium acetate buffer (pH 5.0), whereas the remaining solutions were prepared in 50 mM Tris-HCl buffer (pH 8.0). All chemicals were from Sigma Chemical Company, St. Louis. Sperm treatment consisted of either the control buffers of the experimental solutions at room temperature for 30 min for sperm suspensions or 60 min for sperm slides. The periodate oxidation procedure followed that reported by Woodward.[13] After treatment, the sperm were washed with two changes of the respective buffers and twice with PBS. Air-dried smears of sperm suspensions were prepared on slides, and the slides were stored at 5°C or immediately processed for indirect immunofluorescence and lectin binding as described above.

Sperm-Animal Ovum Penetration Assay

The basic technique used was that described earlier with the following modifications: donor sperm cells after semen liquification were allowed to swim up into 3–4 volumes of Biggers, Whitten, and Whittingham (BWW) medium[14] for 30–60 min, were washed twice with BWW, and were resuspended at 5×10^6 sperm/ml concentration in a 12 × 75-mm plastic tube for overnight incubation (15–17 hs) to induce capacitation.[15] The sperm were diluted to 10^6/ml with sterilely prepared hybridoma medium, cell supernatants, or ascites diluted with BWW to achieve final concentrations of 50% for hybridoma cell media and 10% for ascites. The sperm-MAb mixtures were incubated for 1 hour and washed twice with fresh BWW. Then 200-μl drops were placed under mineral oil in 10 × 35-mm plastic petri dishes and 15–25 zona-free hamster eggs added per sample. After 2.5–3 h incubation, the eggs were washed twice with fresh BWW, fixed for 20 h in 10% phosphate-buffered formalin, and stained with acetolacmoid for observation of bound sperm and swollen sperm heads. Motility was observed for the sperm samples after capacitation, antibody treatment, and coincubation with the ova.

Possible binding of the MAbs with hamster ova was examined by using indirect immunofluorescence. Zona-free ova were incubated with ascites diluted 1:20 with BWW containing 20 mM Hepes buffer for 20 min at room temperature, washed twice by transfer to fresh BWW, reacted with fluorescein-labeled second antibody for 20 min, washed twice, and placed in small volumes of medium under mineral oil on microslides for fluorescent microscopy.

Sperm-Cervical Mucus Penetration Assay

The methodology utilized was that previously reported for antibody-treated sperm and estrous bovine cervical

mucus (CM).[16] Three replicates were run per MAb sample by using different sperm donors. Sperm penetration was the distance achieved in the column of CM by the vanguard group of five motile sperm after incubation for 30 min at 37°C. In our system, control sperm routinely reached the upped end of the CM column (>50 mm) in large numbers by 30 min. The CM Penetration Index was based on the penetration distance of MAb-treated sperm in relation to 50 mm. In addition, the assay was repeated for some of the antibodies on a CM sample obtained at midcycle from a normal ovulatory woman.

Immunohistochemistry

The MAbs were evaluated for sperm specificity by use of the avidin-biotin-immunoperoxidase complex (ABC) method.[17] Cryostat sections of human somatic tissues (spinal cord, liver, kidney, and spleen) and testes were fixed in acetone for 10 min at room temperature, blocked with separate incubations in 0.1% avidin and 0.01% biotin, and treated with 0.3% H_2O_2 in methanol to remove endogenous peroxidase activity. The tissues were then blocked with 25% goat normal serum (GNS) in TBS (0.1 M Tris-buffered saline, pH 7.6) before incubation with MAbs for 2 h. After washing twice in TBS (10 min) biotinlyated goat antimouse Ig (Cappel Labs) at 1:200 dilution in TBS containing 6% GNS was incubated with the sections for 30 min. The sections were washed and then reacted for 30 min with a 1:400 dilution of the ABC reagent (Vectostain). After washing, the peroxidase label was developed by reacting sections for 5 min in the dark with 0.04% diaminobenzidine in TBS containing 0.01% H_2O_2. The sections were then flooded with distilled H_2O for 10 min, counterstained with hematoxylin, washed in H_2O, dehydrated through a series of graded alcohols into xylene, and coverslipped using Permount.

RESULTS

The Ig class and subclass distribution for the 15 MAbs consisted of 13 IgGl, one IgG3, and one IgM (Table I). Twelve of the MAbs were found to be nonreactive with different human somatic tissues (kidney, liver, spinal cord, and spleen) by indirect immunofluorescence (IF) with cell suspensions or by immunoperoxidase staining of cryostat sections of the tissues. The exceptions were 80, 85, and HS-126, which reacted to varying degrees with peripheral blood lymphocytes and spleen tissue as well as strongly with the plasma membranes of live and KCl-washed sperm cells. These three MAbs labeled the plasma membrane in IF and also caused sperm agglutination and complement-dependent immobilization. MAb 16 reacted in IF with the sperm plasma membrane but did not cause agglutination nor immobilization. MAb 218, reactive with the neck of the sperm cell, and MAb 235, an acrosome stainer, also produced complement-dependent immobilization of sperm. Each of the MAbs capable of immobilizing sperm reacted equally well with capacitated sperm in the immobilization assay as with freshly processed ejaculated sperm cells. Significant inhibition of sperm migration through cervical mucus was demonstrated only by MAbs 80, 85, and HS-126, whereas MAbs 235 and 1A5-20 showed slight effects using bovine CM. MAbs 80 and 85 also exhibited the greatest inhibitory effects on sperm penetration of zona-free hamster eggs. In addition, MAbs reactive with acrosomes (11, 63, and 106), tail (97, HS-30), the neck (218), and the nonspecific stainer HS-126 were also effective in reducing the rate of sperm-egg fusion. Sperm motility was not less than 50% in the function assays, with the range being from 50% to 70% among trials at the finish of the experiments. MAb 80, while it was the only antibody to do so, did react in immunofluorescence with the vitellus of the hamster ovum.

Fab preparations made from ammonium sulfate precipitates of acsites of MAbs 11, 80, 85, 235 and control ascites were used in immunofluorescent and functional assays. Each of the Fabs reacted as the native antibodies in IF on sperm, but the agglutinating and complement-dependent immobilizing activities were largely lost. None of the Fabs showed any significant inhibitory effects on sperm in the CM penetration assay nor in the hamster egg assay. The number of ova penetrated per

TABLE I. Reactions Characterizing 15 Different MAbs Against Human Sperm

MAb clone	Ig class	Immunofluorescence live sperm[a]	Antibody Assay TAT	Antibody Titers[b] SIT	Cervical mucus penetration index[c]	Zona-free ovum penetration No. trials	Control sperm No. ova	Control sperm Percent ova penetrated	MAb sperm No. ova	MAb sperm Percent ova penetrated	Penetration index[c]
11	G1	Ac(80)	–	–	100 (100)	4	70	51.4	64	14.1*	27.4
16	G1	PM(30)	–	–	100	3	46	54.3	49	51.0	93.9
33	G1	A(35)	–	–	100	3	45	55.6	54	50.0	89.9
57	G1	T(10)	–	–	100	3	50	60.0	48	62.5	104.2
63	G1	Ac(80	–	–	100 (100)	4	74	54.1	63	11.1*	20.5
80	G1	PM(100)	2,048	1,024	5 (15)	4	64	45.3	56	1.8*	4.0
85	G1	PM(100)	1,024	–	10 (20)	4	64	45.3	63	0.0*	0.0
97	G1	T(20)	–	–	100	4	74	48.9	76	15.8**	32.3
101	G1	T(70)	–	–	100	4	74	55.4	68	36.8	66.4
106	G1	Ac(80)	–	–	100 (100)	4	65	44.6	68	13.2**	29.6
218	G1	N(100)	–	64	100 (100)	3	46	54.3	50	12.0**	22.1
235	G1	A(65)	–	128	75 (100)	3	46	54.3	50	28.0	51.6
1A5-20	M	T(20)	–	–	80 (100)	4	71	59.2	77	31.2	52.7
HS-30	G1	T(90)	–	–	100 (100)	4	80	52.5	61	24.6**	46.8
HS-126[d]	G3	PM(100)	1,024	512	20 (20)	4	62	64.1	62	7.0*	26.4

[a]( ), percent sperm reactive; A, acrosome; Ac, acrosome cap; N, neck; PM, plasma membrane; T, tail.
[b]TAT, agglutination technique; SIT, immobilization technique; –, indicates negative reaction.
[c]Percent of control values; values in parentheses obtained with human CM.
[d]Reactive with lymphocytes.
*P < .01.
**P < .05.

total number of ova and (%) for three trials were as follows: control Fab, 33/47 (70.2%); Fab 11, 29/45 (64.4%); Fab 80, 29/45 (64.4%); Fab 85, 24/47 (51.1%); and Fab 235, 21/36 (58.3%). The activity was not lost during the salting out procedure as the immunoglobulin (Ig) fractions of MAbs 11 and 85 allowed sperm penetration of 2/16 (12.5%) ova and 0/14 ova, respectively, compared with 18/18 (100%) ova for the Ig fraction of control ascites. In addition, sperm treated with the Ig fractions exhibited CM penetration results similar to those seen after treatment with ascites: namely, penetration comparable to control for MAb 11 Ig (>50 mm) and an inhibition for MAb 85 Ig (<5 mm).

Immunofluorescent reactions of MAbs on KCl-washed sperm in comparison with reactions on live sperm showed similar regional staining, but the intensity and percentage of cells stained were greater with antibodies 16, 33, 57, 97, and 1A5-20. The negative controls gave a complete absence of fluorescent labeling of cells. Whereas MAbs 11, 63, and 106 stained the acrosomal cap outline of 80% of the cells, in the live sperm samples with KCl-washed and methanol-fixed sperm 70–80% of the cells showed staining of the whole acrosome. Since the air-dried smears and cell suspensions of KCl-washed sperm had similar reactions to the different treatments, except where noted, only the results on air-dried sperm preparations are presented in Table II. Compared to KCl-treated sperm cells, methanol fixation resulted in less staining reaction in IF with five MAbs. Three of these reacted with the tail (MAbs 57, 101, HS-30), one reacted with the neck (MAb 218), and the nonspecific plasma membrane reactor reacted with HS-126. Methanol fixation also reduced the degree and percentage of acrosomes labeled by MAb 33 but did not appear to affect staining of the equatorial segment. Periodate treatment of KCl-washed sperm completely eliminated labeling of the cells with these same six MAbs and, in addition, with MAb 85, a plasma membrane reactor.

Treatment of the air-dried sperm smears with the detergent NP-40 and chaotropic agent LIS resulted in similar loss of reaction with elimination of plasma membrane staining by MAbs 80, 85, and HS-126; reduction of plasma membrane staining (whole sperm) to only midpiece and tail for MAb 16; a reduction in percentage of acrosomes staining for MAb 63 and complete loss of acrosomal staining for sperm suspensions by MAbs 11, 63, and 235. In addition, LIS treatment of sperm smears resulted in loss of neck staining by MAb 218.

Of the enzyme treatments, neuraminidase appeared to be without effect on the staining patterns by the different MAbs. The proteolytic enzymes, however, showed varied effects on sperm by antibody labeling. Trypsin eliminated acrosome and equatorial binding of MAbs 33 and 235, neck reaction of MAb 218, and the tail labeling by MAb 97. In addition, the strong plasma membrane binding by MAb 16, 80, and 85 was reduced to tails only being bound. On sperm suspensions trypsin showed the same effect as on sperm smears except for MAbs 80, 85, 218, and 235, which gave reactions equivalent to enzyme-untreated KCl-washed sperm. Pronase, similar to trypsin, caused loss of tail staining by MAb 97, neck staining by MAb 218, and a reduction of membrane staining to tail only by MAbs 80 and 85. Chymotrypsin and pronase generally exerted the same effects on sperm cell binding by the different MAbs with elimination of reactions with antibodies 11, 16, 33 (reduced), and 85. Again, these two enzymes were without effect on sperm suspensions for MAbs 11 and 85, which had reactions not different from KCl treatment only.

Studies with fluoresceinated Con A revealed that lectin binding to the plasma membrane was greatest on the head of the sperm cell with strong staining of the equatorial segment, postacrosomal area and neck, whereas the entire tail (midpiece and principal piece) was labeled to a lesser extent. The labeling of the plasma membrane and neck was eliminated by treatment of sperm with detergent, methanol, and LIS before exposure to Con A. Sperm treated with periodate (5–20 mM) failed to bind any Con A, whereas sperm exposure to the proteolytic enzymes or neuraminidase did not result in any detectable loss of Con A binding compared with appropriate control sperm.

Immunocytochemistry revealed that all 15 MAbs analyzed bound to testicular sperm compared with control

TABLE II. Monoclonal Antibody Binding Observed With Indirect Immunofluorescence on Air-Dried KCl-Washed Human Sperm Subjected to Different Treatments*

Antibodies	KCl	Methanol	Periodate	NP-40	LIS	Trypsin	Chymotrypsin	Pronase	Neuraminidase
11	A	A	A	A[a]	A[c]	A	–[b]	–[b]	A
16	PM	PM	PM	Mp,T	Mp,T	T	–	–	PM
33	A,ES	ES(A)	–	–	–	–	(A,ES)	(A,ES)	A,ES
57	T	–	–	T	T	T	T	T	T
63	A	A	A	(A)[a]	(A)[a]	A	A	A	A
80	PM	PM	PM	–	–	T[c]	PM	T[b]	PM
85	PM	PM	–	–	–	T[c]	(T)[b]	(T)[b]	PM
97	T	T	T	T	T	–	T	–	T
101	T	–	–	T	T	T	T	T	T
106	A	A	A	A	A	A	A	A	A
218	N	–	–	N	–	–[b]	N	–[b]	N
235	A	A,ES	A	A[a]	A[a]	–[b]	A	A	A,ES
1A5-20	MP,T	MP,T	MP,T	MP,T	MP,T	MP,T	MP,T	MP,T	MP,T
HS-30	T	–	–	T	T	T	T	T	T
HS-126	PM	–	–	–	–	PM	PM	PM	PM

*Abbreviations: A, acrosome; ES, equatorial segment; MP, midpiece; N, neck; PM, plasma membrane; T, principal tail piece; ( ), intensity and/or percent cells staining reduced by one-half or more; –, negative.
[a]Staining of sperm suspension was negative.
[b]Staining of sperm suspension was positive and equal to KCl-only treatment.

TABLE III. Monoclonal Antibody Binding Observed With Indirect Immunofluorescence on Murine and Rabbit Sperm Cells After Different Treatments*

	Murine sperm				Rabbit sperm			
Antibodies	Live	KCl	Methanol	Periodate	Live	KCl	Methanol	Periodate
11	Ac	A	A	A	Ac	A	A	A
33	A	A	A	(A)	A	A	A	A
63	Ac	A	A	A	Ac	A	A	A
106	Ac	A	A	A	Ac	A	A	A
218	N	N	–	–	N	N	–	–
235	Ac	A	A	A	A	A,ES	A,ES	A
1A5-20	PM	PM	PM	PM	PM	PM	PM	PM

*Abbreviations from Tables I and II.

ascites and culture medium, which gave negative results. The staining patterns we obtained were generalized on the tail, postacrosomal area, or whole head without specific labeling of the acrosome. The testis results were generally consistent with those obtained by immunofluorescence on ejaculated sperm in terms of the structure and percentage of cells labeled, with the following exceptions observed on the testis sections: acrosome-staining antibodies labeled the whole sperm head; MAb 97 labeled the entire sperm cell; and MAb 218 stained the postacrosomal region near the neck in approximately 25% of the sperm cells. Reaction with the spermatids, especially the late stages, was observed with MAbs 11, 63, 85, 106, 218, 235, HS-30, and HS-126. Because cell morphology of the earlier stages of spermatogenesis was poorly preserved, we could not determine accurately the earliest stages at which antibody binding occurred.

Of the 15 MAbs evaluated, seven (11, 33, 63, 106, 218, 235, and 1A5-20) reacted in indirect immunofluorescence with the sperm cells of mouse and rabbit (Table III). Methanol fixation and periodate oxidation of the animal sperm gave results similar to those observed with human sperm after application of the MAbs in immunofluorescence.

DISCUSSION

Regional heterogeneity of the sperm plasma membrane and infrastructure has been well documented by using antibodies,[9,18,19] lectins,[20] and other labels.[21] Antigenic determinants on sperm cells should be components of molecules of proteins, glycoproteins, and glycolipids as on other types of cells.[22] Incorporating the different techniques with monoclonal antibodies allows characterization of sperm antigens and their possible involvement in immunologic infertility. Earlier we reported that subjecting human sperm to capacitating conditions increased the binding of some MAbs while decreasing the binding of other MAbs.[23] Brief exposure of ejaculated sperm to a hypertonic solution of 0.5 M KCl, which is known to remove peripheral proteins and glycoproteins from cells, resulted in MAb-binding patterns similar to those observed after capacitation. KCl treatment, in comparison to capacitation, however, resulted in an even and consistent regionalized staining of sperm. MAb 57, for example, showed binding to a small percentage of the tails of live sperm, binding increased to 60–80% of the tails of capacitated sperm but with patchy fluorescence, whereas 100% of the tails of KCl-treated sperm exhibited even and bright fluorescence. Therefore, for this study, KCl-washed sperm were utilized.

Mild periodate oxidation of antigens that cause cleavage of the carbohydrate vicinal hydroxyl groups was shown to be a reliable method to detect anticarbohydrate monoclonal antibodies.[13] The combined immunofluorescent results on sperm obtained after periodate and methanol treatments suggests that three of five MAbs reactive with the tail as well as MAbs 218 (neck) and HS-126 (plasma membrane) were against carbohydrate epitopes on glycolipids. Use of the solvent n-pentanol in place of methanol on sperm smears and suspensions also resulted in loss of tail, neck, and plasma membrane reactions to these MAbs.

The remaining two periodate-sensitive epitopes detected by MAbs 33 and 85 appeared to be on glycoprotein molecules as they also were affected by proteolysis. Lectin-binding studies confirmed the nature of the glycolipid antigens as reactions with the plasma membrane and neckpiece were absent from sperm treated with periodate and organic solvents. The glycolipid components of the tail appeared resistant to removal by detergent and the chaotropic agent, LIS, suggesting that the molecules may be anchored in the infrastructure.

The acrosomal antigens detected by immunofluorescence with the MAbs appear to be complex molecules that contain carbohydrate and peptide epitopes. The antigen detected by MAb 33 appeared to be readily released or destroyed after probable dissolution of the plasma and outer acrosomal membranes by detergent and LIS, whereas the antigen detected by MAb 106 seemed unaffected by any of the treatments. Antigen 106, or at least the epitope recognized by MAb 106, may be expressed both externally as well as internally on acrosomal structures.

Since none of the reactions of MAbs was diminished nor increased by neuraminidase, it suggests that sialic acid was not directly involved with any of the antigenic determinants.

Recent evidence in some species suggests that gamete interactions involve lectin-ligand type interactions.[24,25] These interactions have been inhibited by treating eggs with different sugars, simple to complex, which presumably block receptor-like molecules on the egg surface specific for plasma membrane components of sperm.[24,26] Since these components would be glycosylated proteins and lipids, it was of interest to determine if antibodies reactive with carbohydrate epitopes as well as peptide epitopes would affect sperm function in the fertilization process.

Results of the antibody inhibition assays suggest that of the selected clones we evaluated, antibodies 80, 85, and HS-126, which were reactive against antigens of lymphocytes as well as the plasma membrane as indicated by agglutination and immunofluorescent methods, were most effective in blocking sperm function. In addition, MAb 80 was reactive with the vitellus of the hamster ovum. Since in the sperm penetration assay, however, the sperm were washed twice after incubation with antibody, it is unlikely that there was adequate free antibody remaining in the samples to bind effectively to the ova and to block sperm fusion. The inhibition of sperm penetration of cervical mucus and possibly also of hamster ova was likely due to the agglutination activity of the antibodies, although it was estimated by microscopic examination at the time of assay evaluation that at least 10% of the sperm present were free swimming. The failure of Fab preparations of the MAbs 80 and 85 to inhibit sperm activity in these assays would support this conclusion, especially for the cervical mucus assay. The inhibition of sperm penetration of zona-free eggs occurring with other intact MAbs that were nonagglutinating and that failed to inhibit when used as Fab preparations would suggest, however, that the observed effect was due neither to agglutination per se nor to blocking of a specific sperm-membrane receptor molecule for the oocyte vitellus but was possibly a result of steric hindrance. Two recent reports on inhibition of sperm fusion with hamster ova used MAbs that also did not cause sperm agglutination nor affect motility.[6,7] The one MAb raised against human sperm cells reacted with the postacrosome, midpiece, and tail regions of sperm and was highly effective in preventing sperm binding to the egg membrane with a resultant lack of gamete fusion,[6] whereas the other MAb raised against a rabbit sperm antigen (RSA-1) reacted with the midpiece of human sperm and caused a reduced penetration rate but did not affect sperm binding to the vitellus.[7] The antigens recognized by these two MAbs were reported to be glycoproteins, although the nature of the epitopes was not determined.

Except for the three MAbs strongly reactive with plasma membrane antigens, significant effects on sperm penetration of cervical mucus were not observed with the other MAbs. In addition to these three MAbs we did find that other MAbs reactive with the acrosome, neck, and principal piece of the tail produced significant inhibition of sperm fusion with hamster ova. The mechanism(s) by which antibodies against antigens of the sperm neck and tail inhibit membrane fusion and swollen head formation is unclear, although the results are repeatable with isolated immunoglobulin fractions from the MAbs, which suggests that nonspecific effects are not a factor (unpublished data). These active MAbs were apparently against the carbohydrate epitopes on glycolipids as well as on glycoproteins or the peptide epitope of proteins. Comparison of immunofluorescent results on sperm of man, mouse, and rabbit suggests a conservation of both certain carbohydrate and peptide epitopes among these three species. Specific glycolipid compounds have been identified in sperm of man and other mammalian species.[27,28] In mice, the highly immunogenic molecules of teratocarcinoma cells are glycolipids and may have played a role in induction of infertility in females immunized with the tumor cells.[29,30] The murine sera against the teratocarcinoma cells reacted positively with murine sperm,[31] and in a study of human infertility cases approximately one half of the sera positive for sperm agglutinating antibodies but none of the negative sera reacted with murine F9 teratocarcinoma cells.[32] These reports and our data on inhibition of sperm penetration of zona-free ova for the two MAbs (218 and HS-30) presumably reacting with glycolipid components of sperm suggest that these molecules may play a role in the fertilization process and that antibodies against glycolipids as well as glycoproteins may produce infertility. That some of these epitopes of human sperm are also present on sperm of laboratory animals will allow for experimental analyses of their possible functional roles and the antifertility effects of induced immune reactions against the molecules.

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