A Role for the Migrating Sperm Surface Antigen PH-20 in Guinea Pig Sperm Binding to the Egg Zona Pellucida

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ABSTRACT After the acrosome reaction, the PH-20 surface antigen of guinea pig sperm migrates from its original location on the posterior head surface to a new location on the inner acrosomal membrane (Myles, D. G., and P. Primakoff, 1984, *J. Cell Biol.*, 99: 1634–1641). We have isolated three monoclonal antibodies (MAbs) of the IgG1 subclass, PH-20, PH-21, and PH-22, that bind to the PH-20 antigen. The PH-20 MAb strongly inhibited (~90%) sperm binding to the guinea pig egg zona pellucida at saturating antibody concentrations (>20 μg/ml). Half-maximal inhibition of sperm binding to the zona was obtained with ~2 μg/ml PH-20 MAb. The PH-21 MAb at saturating concentration (50 μg/ml) partially inhibited (~45%) sperm-zona binding, and the PH-22 MAb (50 μg/ml) did not inhibit (0%) sperm-zona binding. Essentially the same amounts of the three MAbs were bound to sperm under the conditions where inhibition (PH-20, PH-21) or no inhibition (PH-22) of sperm-zona binding was observed, which indicates that the different levels of inhibition did not arise from different levels of MAb binding. Competition binding assays with ^{125}I-labeled MAbs showed that PH-21 binding to sperm was not affected by the binding of PH-20 or PH-22. However, that PH-20 and PH-22 blocked each other's binding to sperm suggests that their recognized determinants may be relatively close to one another. The results indicate that the migrating PH-20 antigen has a required function in sperm binding to the zona pellucida and that the PH-20 MAb affects its active site.

During mammalian fertilization, male and female gametes traverse long distances in the female reproductive tract. Upon encountering each other in the oviduct, they recognize one another and bind. At the phenomenological level, mammalian gamete recognition is known to be relatively species specific, to occur between the sperm surface and the egg zona pellucida, and to involve a tenacious yet reversible binding. The binding appears to be reversible, since some bound sperm proceed from the surface of the zona pellucida to penetrate through the zona and reach the perivitelline space.

At the molecular level, only limited insights into the mechanisms of mammalian gamete recognition have been obtained. In the mouse, evidence has been presented that sperm have a surface galactosyltransferase activity which is required for sperm-zona pellucida binding (22, 23). This surface enzyme can bind to terminal *N*-acetylglucosamine residues on zona pellucida glycoproteins to promote sperm-egg adhesion. It has also been reported in the mouse that ZP-3, one of the three zona glycoprotein constituents, can specifically inhibit sperm-egg binding (1, 2, 5). This inhibitory ability of ZP-3 suggests it may be a receptor for sperm on the zona pellucida of the mouse egg. In other mammalian species, molecular hypotheses about gamete recognition cannot yet be formulated.

An aspect of gamete recognition that remains to be investigated is the role of surface topography in the binding process. It is well established that in the polarized sperm cell many of the surface proteins are restricted to particular domains (7, 20). In addition, it has recently been found that either before or during sperm-zona pellucida binding, some of these proteins migrate to new domains (14). The zona pellucida may also have a nonhomogeneous distribution of certain components from outside to inside (3, 15), and this too might contribute to the properties of sperm-egg adhesion.

In the current paper, we report experiments on sperm-zona pellucida binding with guinea pig gametes. Our results suggest that the PH-20 antigen, a sperm surface protein that migrates to a new domain after the acrosome reaction, has a role in sperm binding to the zona pellucida. The findings thus identify a candidate for a surface antigen of guinea pig sperm with

an essential function in sperm-zona binding. Since this antigen undergoes a migration, the dynamics of cell surface localization may contribute to the process of sperm-egg adhesion.

MATERIALS AND METHODS

Animals: Hartley guinea pigs were obtained from Buckberg Lab Animals (Tomkins Cover, NY). Males were larger than 700 g and females were in the range of 275–350 g.

Reagents: Fucoidin was obtained from Sigma Chemical Co. (St. Louis, MO). The Bolton-Hunter reagent, 4,000 Ci/mM, used to label monoclonal antibodies (MAbs)[1] with ^{125}I, was from New England Nuclear (Boston, MA). ^{125}I-Labeled second antibodies were ^{125}I-sheep anti-mouse IgG and ^{125}I-rabbit anti-mouse IgG from New England Nuclear. Second antibodies specific to particular subclasses of mouse immunoglobulins for use in Ouchterlony assays were from Meloy Laboratories Inc. (Springfield, VA). Goat IgG anti-mouse IgG coupled to Sepharose CL-4B was from Cooper Biomedical Inc. (Malvern, PA). Fluorescently labeled second antibodies were rhodamine-conjugated goat F(ab')$_2$ anti-mouse IgG or goat Fab anti-mouse IgG, also from Cooper Biomedical Inc.

MAbs: The hybridoma lines producing antibodies to the guinea pig cell surface have been described (20). They were generated by fusions between spleen cells from immunized C57-B1/6 female mice and the myeloma line P3-NS1/Ag4-1. The lines were cloned by limiting dilution as previously described (13, 20).

MAbs were isolated from culture supernatant before use in the sperm-zona binding assay. The antibodies were concentrated by $(NH_4)_2SO_4$ precipitation and purified on DEAE-cellulose (11, 17). A linear gradient from 0 to 200 mM NaCl was run after MAb was applied to the column. Fractions containing peak activity of the PH-20 MAb eluted at 50 mM NaCl, peak activity of the PH-21 MAb eluted at 121 mM NaCl, and peak activity of the PH-22 MAb eluted at 55 mM NaCl. Peak fractions were concentrated by ultrafiltration, dialyzed into phosphate-buffered saline, and frozen. On SDS gel electrophoresis, the DEAE-isolated antibodies appeared to be between 50–80% pure. Antibody subclass was determined by Ouchterlony assays (11). Antibody concentrations were measured by single radial immunodiffusion as previously described (14).

To produce ^{125}I-labeled MAbs, DEAE-isolated antibodies were reacted on ice with the ^{125}I-Bolton-Hunter reagent following the supplier's instructions. ^{125}I-Conjugated antibody was separated from unreacted reagent on a Sephadex G-50 column.

Immunoprecipitation: Cell surface labeling with ^{125} followed by detergent extraction, immunoprecipitation, SDS PAGE, and autoradiography were performed as previously described (13, 20). To preclear ^{125}I-labeled detergent extracts with the PH-22 MAb, 40 μl of a 50% suspension of Sepharose beads coupled to goat IgG anti-mouse IgG was incubated overnight at 4°C on a rotator with 0.8 ml PH-22 culture supernatant. The beads were washed three times by low speed centrifugation and resuspended as a 50% suspension in Triton solubilization buffer (20). 20 μl of the resuspended beads were added to 4×10^6 cpm of ^{125}I-labeled detergent extract of sperm in 0.6 ml Triton solubilization buffer, and incubated for 1.5 h at 4°C on a rotator, and then the beads were pelleted. To the supernatant, an additional 20 μl of PH-22 incubated beads was added, and a second 1.5 h incubation was performed. The beads were again pelleted, and the supernatant, which is the precleared extract, was split into four aliquots for immunoprecipitation with PH-20, PH-21, PH-22, and the control, NS-1 culture supernatant + 100 μg/ml mouse IgG.

Indirect Immunofluorescence: Indirect immunofluorescence was performed as previously described (13, 20). Second antibodies used were rhodamine-goat F(ab')$_2$ anti-mouse IgG or rhodamine-goat-Fab anti-mouse IgG.

In Vitro Sperm-Zona Binding: The sperm-zona binding assay was performed as detailed by Huang et al. (9, 10). Sperm were removed from the cauda epididymis into 0.9% NaCl at 1×10^8 cells/ml, and 100-μl aliquots were diluted into 1.4 ml of modified Tyrode's medium without Ca^{2+} (mT − Ca^{2+}) containing 70–100 μg/ml lysophosphatidyl choline (Sigma Chemical Co.). The sperm were incubated under mineral oil at 37°C for 1 h, and 1.5 ml mT + 4 mM $CaCl_2$ was added to stimulate the acrosome reaction. 5–10 min later, 100 μl of the acrosome-reacted sperm suspension was mixed with 100 μl mT + 2 mM $CaCl_2$ (control) or 100 μl DEAE-isolated MAb (dialyzed into mT + 2 mM $CaCl_2$). The concentration of MAb in the 200 μl mixture with sperm ranged from 1 to 50 μg/ml. Incubation of sperm with MAb was continued for 15–20 min at room temperature. 70 μl of the sperm-antibody mixture was added to a 280-μl drop of mT + 2 mM $CaCl_2$ containing eggs. The eggs had been prepared according to Yanagimachi (26) by removal from the ovaries, overnight culturing, and removal of the cumulus cells with hyaluronidase. Sperm and eggs were incubated at 37°C for 30 min (11), the eggs were removed from the insemination drop with a wide-bore micropipette, placed in a drop containing 2.5% glutaraldehyde, and washed, and the sperm bound per egg were counted in a single plane of focus.

Measurement of Levels of Antibody Bound to Sperm in the Sperm-Zona Binding Assay: Sperm were capacitated, acrosome reacted, and incubated with 25 μg/ml DEAE-isolated MAb as just described for the sperm-zona binding assay. The sperm-antibody mixture was diluted into 4 vol mT + 2 mM Ca^{2+} and incubated for 30 min more at 37°C, as was done when eggs were present in the sperm-zona binding assay. The sperm were then pelleted, incubated with ^{125}I-labeled second antibody, pelleted again, and counted in a gamma counter.

Competition Binding of ^{125}I-labeled MAbs: To detemine if the MAbs competed with one another for binding to sperm, competition binding assays were performed using modifications of the protocols of Kohler (11) and Stahli et al. (24). 5×10^6 live sperm (acrosome intact or acrosome reacted) in 100 μl medium were incubated for 2 h at room temperature with 50 μg/ml unlabeled MAb. The ^{125}I-labeled MAb (10 μl at 2 μg/ml) was added, and incubation continued for 1 h at room temperature. The sperm-antibody mixture was layered over 1 ml horse serum, and the sperm were pelleted, resuspended in 200 μl phosphate-buffered saline, and counted in a gamma counter.

RESULTS

Previously we isolated a collection of MAbs to surface antigens of guinea pig sperm (13, 20). In initial experiments, we surveyed many of these antibodies for possible effects on sperm binding to the zona pellucida of guinea pig eggs. The PH-20 MAb in dialyzed culture supernatant was found to inhibit sperm binding. We therefore decided to study more closely the effect of this antibody and to look at the effects on sperm-zona binding of other antibodies to the same antigen.

Antibodies to the PH-20 Antigen

The PH-20, PH-21, and PH-22 MAbs bound to the posterior head surface of acrosome-intact guinea pig sperm (20). From detergent extracts of ^{125}I surface-labeled sperm, each of the three antibodies precipitated the same antigen. In our nomenclature, this antigen is named the PH-20 antigen (20). On nonreducing SDS polyacrylamide gels, the PH-20 antigen runs as a diffuse, broad band, possibly a doublet, with M_r ~59,000–66,000 (Fig. 1 *A*, lanes *1–3*, showing PH-20, PH-21, and PH-22 immunoprecipitates, respectively). The antigen was not precipitated in the control (Fig. 1 *A*, lane *4*). A second experiment to test if the three antibodies recognized the same antigen was to preclear the ^{125}I-labeled extract by precipitation with one antibody and to ask if any material remained in the precleared extract that could be precipitated by the other antibodies. After preclearing with the PH-22 MAb, the PH-20, PH-21, and PH-22 MAbs precipitated only a trace amount of the M_r ~59,000–66,000 antigen (Fig. 1 *A*, lanes *5–7*), again indicating that the three antibodies recognize the identical antigen. The immunoprecipitates of all three antibodies run on reducing SDS PAGE were the same and showed two bands with M_r ~41,000 and ~48,000 and a trace-labeled band with M_r ~59,000–66,000. Both one-dimensional peptide mapping and attempts to block more completely proteolysis during the procedures indicate that the ~41,000 and ~48,000 bands may be derived by proteolytic cleavage from the ~59,000 and ~66,000 bands. Further investigation of this possibility and other aspects of PH-20 antigen structure are being pursued.

Antibody Inhibition of Sperm-Zona Binding

In Ouchterlony analysis, the PH-20, PH-21, and PH-22

[1] *Abbreviations used in this paper:* MAb, monoclonal antibody; mT, modified Tyrode's medium.

MAbs were each found to be of the IgG1 subclass. The three antibodies were isolated from culture supernatant by DEAE-cellulose chromatography (11, 17) and tested for their ability to block sperm binding to the zona pellucida. Sperm were capacitated, Ca^{2+} was added to induce the acrosome reaction, and 5–10 min later antibody was added to the sperm suspension. Incubation was continued for 15–20 min, and then an aliquot of the sperm-antibody mixture was diluted fivefold into a drop containing eggs. After 30 min of incubation at 37°C, the number of sperm bound per egg was scored. Results obtained are illustrated in Fig. 2. In the control, where no antibody was added to the sperm, typically many sperm attached to the zona of an egg (Fig. 2*A*). When sperm were preincubated with the PH-20 MAb, zero or very few sperm bound (Fig. 2*B*).

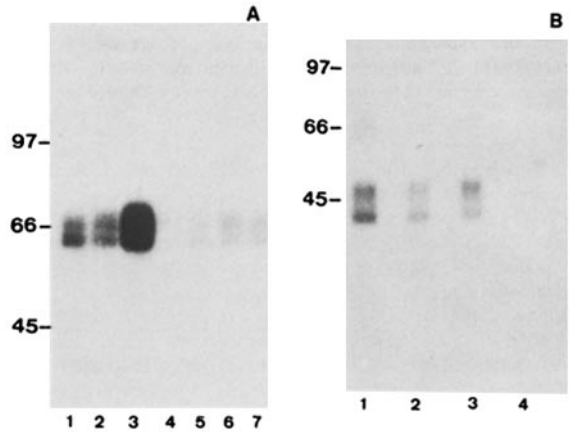

FIGURE 1 Immunoprecipitates of ^{125}I-labeled antigen from Triton extracts of sperm. (*A*) Nonreducing SDS PAGE, 8.5% polyacrylamide gel. Lane *1*, PH-20 MAb; lane *2*, PH-21 MAb; lane *3*, PH-22 MAb; lane 4, control, NS-1 supernatant with 100 μg/ml mouse IgG added. Lanes *5–7*, the ^{125}I-labeled Triton extract was precleared with the PH-22 MAb; lane 5, PH-20 MAb; lane 6, PH-21 MAb; lane *7*, PH-22 MAb. 10^6 cpm of ^{125}I-labeled extract were added for each immunoprecipitate, lanes *1–7*. (*B*) Reducing SDS PAGE, 10% polyacrylamide gel. Lane *1*, PH-20 MAb; lane *2*, PH-21 MAb; lane *3*, PH-22 MAb; lane 4, control, NS-1 supernatant with 100 μg/ml mouse IgG added. 5×10^5 cpm ^{125}I-labeled extract were added for the immunoprecipitate in lane *3*, and 10^6 cpm were added for the immunoprecipitates in lanes *1*, *2*, and *4*. Standards are run under reducing conditions and include myosin, $M_r \sim 205{,}000$; β-galactosidase, $M_r \sim 116{,}000$; phosphorylase B, $M_r \sim 97{,}000$; bovine serum albumin, $M_r \sim 66{,}000$; ovalbumin, $M_r \sim 45{,}000$; and carbonic anhydrase, $M_r \sim 30{,}000$.

Table I shows the data from an experiment in which the PH-20 MAb and the PH-22 MAb were compared for their effect on sperm binding. Sperm were incubated with each MAb at 50 μg/ml and then mixed with eggs. At this concentration, the PH-20 MAb inhibited 90% and the PH-22 MAb did not inhibit. In a series of 12 experiments, PH-20 and PH-22 were retested at 50 μg/ml and other concentrations, and the PH-21 MAb was also tested. The combined data from these experiments are presented in Fig. 3. The PH-20 MAb and the PH-21 MAb both inhibited sperm-zona binding in a concentration-dependent fashion. At saturating concentrations (>20 μg/ml), PH-20 inhibited ~90% and PH-21 inhibited ~45%. Half-maximal inhibition of sperm binding to the zona was obtained with ~2 μg/ml PH-20 MAb. The PH-22 MAb did not inhibit (0%) sperm binding (Fig. 3).

Since the PH-22 MAb did not inhibit, it served as a control antibody for the inhibition found with the PH-20 MAb.

TABLE I. *MAb Inhibition of Sperm Binding to the Egg Zona Pellucida*

Addition	No. of eggs	No. of sperm bound/egg	% inhibition
None	12	14.8	—
50 μg/ml PH-20	13	1.5	90
50 μg/ml PH-22	13	17.8	0

Capacitated sperm were induced to acrosome react, incubated with the antibody concentration shown, and then diluted and mixed with the eggs. After 30 min the eggs were removed, fixed, and scored for number of sperm bound. The data are from one experiment, which was repeated to compile the data in Fig. 3.

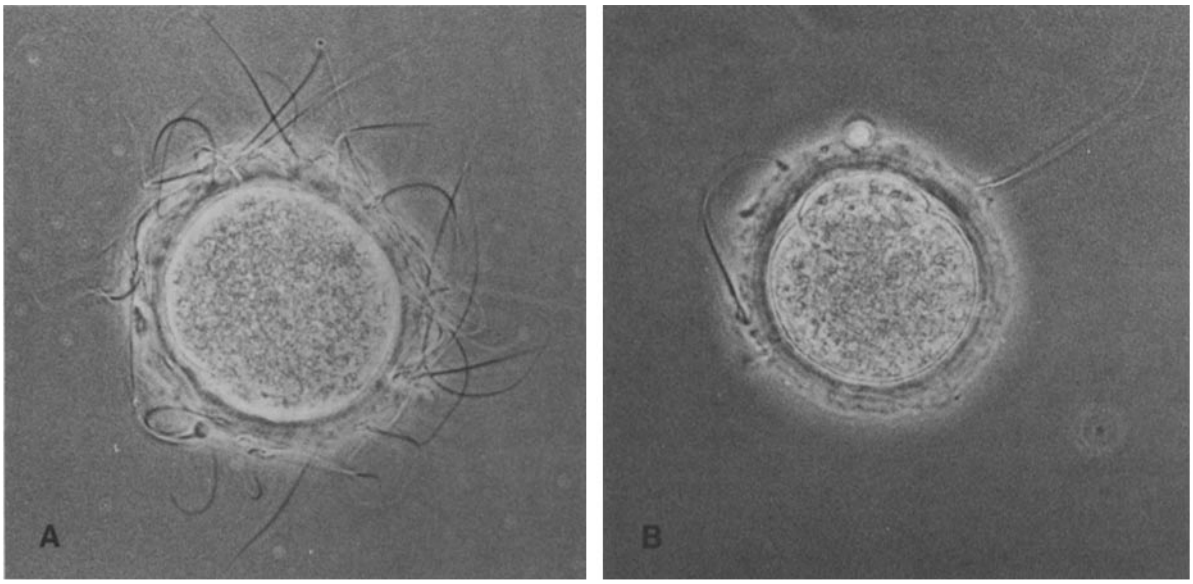

FIGURE 2 Phase-contrast micrographs of eggs from the sperm-zona binding assay. (*A*) An egg from a control dish. 100 μl sperm was incubated with 100 μl modified Tyrode's medium + 2 mM $CaCl_2$ for 15 min before being added to eggs. (*B*) An egg from a dish receiving PH-20 MAb incubated sperm. 100 μl sperm was incubated with 100 μl PH-20 MAb (50 μg/ml) for 15 min before being added to eggs. × 320.

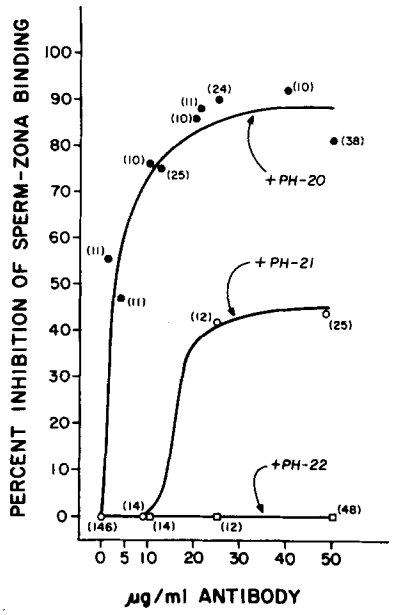

FIGURE 3 Percent inhibition of sperm-zona binding observed over a range of MAb concentrations tested in 12 experiments. 100 µl capacitated sperm, induced to acrosome react by Ca^{2+} addition, was incubated with 100 µl MAb for 15–20 min. In the 200 µl mixture, MAb concentration varied from 1 to 50 µg/ml. In the control, 100 µl sperm was incubated with 100 µl mT + 2 mM $CaCl_2$ for 15–20 min. MAb-treated control sperm were added to eggs and after 30 min of incubation eggs were fixed and sperm bound per egg were counted. The number in parentheses at each data point is the number of eggs scored at that concentration of antibody.

Several other types of control experiments were performed. To test if the PH-20 MAb is inhibiting by some action on the eggs, eggs were preincubated for 30 min with the highest concentration of PH-20 MAb to which they are exposed (10 µg/ml) and then washed and mixed with sperm. No inhibition of sperm binding was seen. Other sperm functions were unaffected by the PH-20 antibody: At concentrations where the PH-20 MAb strongly inhibited sperm-zona binding, we found it did not inhibit sperm fusion with zona-free eggs. Also, none of the tested MAbs had any discernible effect on sperm motility. Three separate preparations of the PH-20 MAb by DEAE-cellulose chromatography were found to inhibit strongly sperm-zona binding. Since the three tested MAbs to the PH-20 antigen each eluted from DEAE-cellulose at a different salt concentration (Materials and Methods), it was conceivable that specific contaminants from hybridoma culture supernatant would co-elute with the PH-20 MAb (but not with PH-21 or PH-22) and contribute to the high inhibition seen with the PH-20 antibody. To test this, culture supernatant from the parental NS-1 myeloma line was passed over DEAE cellulose, and fractions eluting at the same NaCl concentration as the peak PH-20 MAb fractions were pooled. These pooled fractions did not inhibit the sperm-zona binding assay.

Localization and Quantitation of Antibody Binding to Sperm Under Conditions of the Sperm-Zona Binding Assay

In the sperm-zona binding experiments, the MAbs were added to capacitated sperm after Ca^{2+} was added and thus were binding to a mixed population of acrosome-intact and acrosome-reacted cells. The acrosome reaction occurs in 40–80% of the cells under these conditions (references 4 and 9, and our observations). On acrosome-intact cells in the capacitating medium, fluoresence was observed on the posterior head (Fig. 4, *A–F*). After the acrosome reaction, the PH-20 antigen migrates from the sperm's posterior head surface to the inner acrosomal membrane (14). On acrosome-reacted cells in the capacitating medium, fluorescence was observed on the inner acrosomal membrane with the DEAE-isolated PH-20, PH-21, and PH-22 antibodies (Fig. 4, *G–L*).

TABLE II. *Levels of MAbs Bound to Sperm Under Conditions of the Sperm-Zona Pellucida Binding Assay*

	Counts per minute bound	
[MAb]	2.4×10^5 sperm	4.8×10^5 sperm
25 µg/ml PH-20	198,038	340,743
25 µg/ml PH-21	181,308	318,115
25 µg/ml PH-22	215,874	375,384

The values for counts per minute ^{125}I-second antibody bound are the averages of duplicate determinations each done in two experiments. Background counts per minute obtained with sperm and no first antibody have been subtracted. Counts per minute bound to 4.8×10^5 sperm were roughly twofold higher than for 2.4×10^5 sperm, showing that ^{125}I-second antibody was present in excess.

Although the localization of binding of the three antibodies is the same on the sperm used in the zona binding assay, it seemed possible that different levels of binding of the three MAbs occurred, leading to different levels of inhibition. To test this, sperm were capacitated, acrosome reacted, and incubated with MAbs under the conditions of the zona binding assay. The level of MAb bound to the cells was then determined by incubation with an ^{125}I-labeled anti-mouse second antibody. Essentially the same amount of antibody bound to the sperm with each of the three monoclonals; compared with the PH-20 MAb, the partially inhibiting PH-21 MAb showed 8% fewer counts per minute bound, and the noninhibiting PH-22 MAb showed 9% more counts per minute bound (Table II).

Binding of the MAbs in Competition Assays

To gain more information about the arrangement of the three MAb binding sites on the PH-20 antigen, binding assays were done to test the ability of each MAbs to compete with the others for binding to the sperm surface. Live sperm were first incubated with a high concentration of unlabeled MAb and subsequently with an ^{125}I-labeled MAb, and counts per minute bound were determined (11, 24). It was found that PH-20 and PH-22 completely inhibited each other's binding to sperm; PH-21, on the other hand, did not inhibit the binding of either PH-20 or PH-22, nor was the binding of PH-21 affected by the other two MAbs (Table III). The competition between antibodies was found to be the same on acrosome-intact and acrosome-reacted cells. Possible explanations of the competition between the antibodies and their relative abilities to inhibit sperm-zona binding are discussed below.

Effect of Fucoidin on PH-20 MAb Binding

Since Huang et al. (10) have suggested that a sperm surface fucoidin-binding molecule is involved in guinea pig sperm-zona attachment (10), we tested the ability of saturating levels of fucoidin to inhibit binding of the PH-20, PH-21, and PH-22 MAbs. First, we confirmed the observations of Huang et

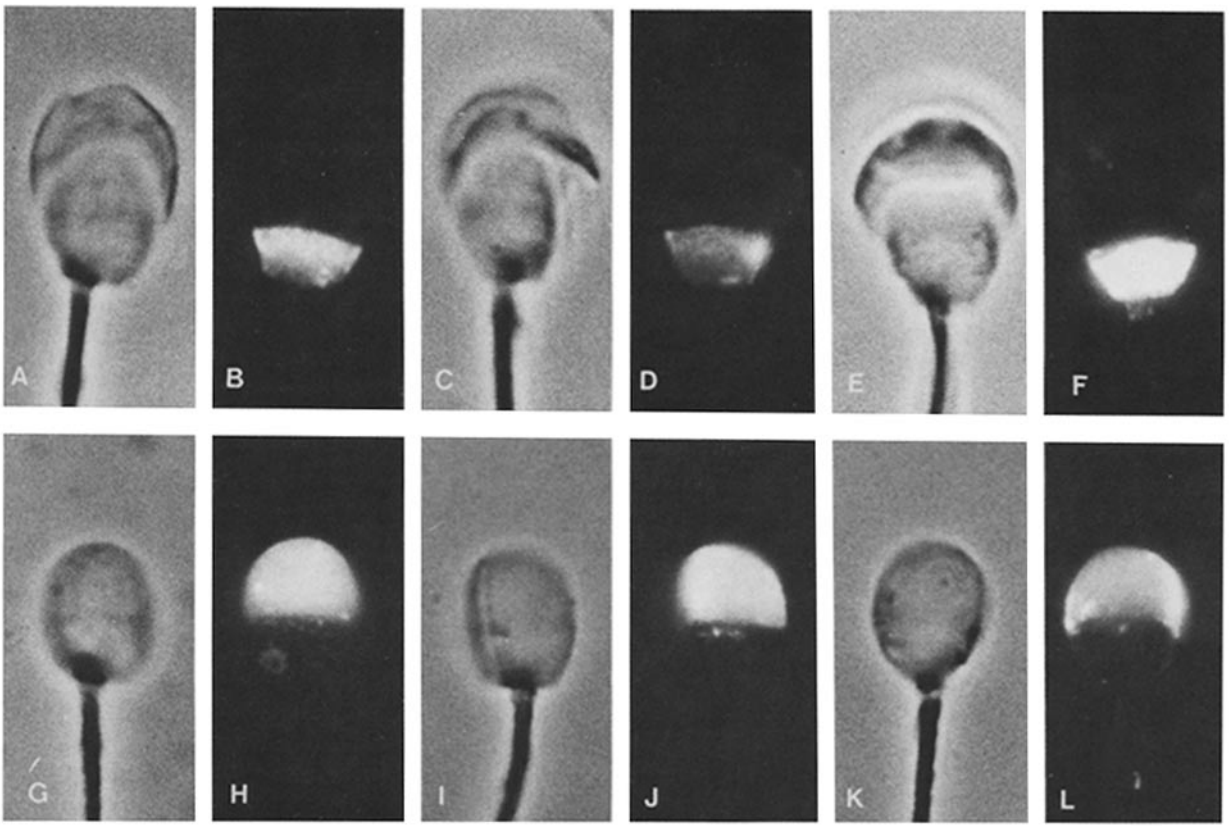

FIGURE 4 Localization of antibody binding on acrosome-intact and acrosome-reacted sperm. Sperm in the capacitating medium were incubated with MAb and a rhodamine-conjugated anti-mouse IgG second antibody was added. (*A–F*) Acrosome-intact cells, fluorescence and corresponding phase-contrast micrographs of the same cell. (*A* and *B*) PH20 MAb; (*C* and *D*) PH-21 MAb; (*E* and *F*) PH-22 MAb. (*G–L*) Acrosome-reacted cells, fluorescence and corresponding phase-contrast micrographs of the same cell. (*G* and *H*) PH-20 MAb; (*I* and *J*) PH-21 MAb; (*K* and *L*) PH-22 MAb.

TABLE III. *Competition Between ^{125}I-labeled MAbs in Binding to Sperm*

Addition	% Binding of ^{125}I-labeled MAb		
	^{125}I-PH-20	^{125}I-PH-21	^{125}I-PH-22
Control (no addition)	100	100	100
50 μg/ml unlabeled PH-20	0	100	3
50 μg/ml unlabeled PH-21	91	0	100
50 μg/ml unlabeled PH-22	0	100	0

Live sperm were incubated for 2 h with 50 μg/ml unlabeled antibody. The cells were then incubated for an additional 1 h with ^{125}I-antibody at 0.18 μg/ml and various specific activities in different experiments. In the control (no unlabeled antibody added) 17,000–200,000 cpm bound depending upon the specific activity of the ^{125}I-antibody used. When the identical unlabeled and ^{125}I-antibody were added, counts per minute bound were five–10-fold lower than the control. Data are the average of experiments done with acrosome-intact and acrosome-reacted cells.

TABLE IV. *Binding of Antibodies to the PH-20 Antigen After Prebinding of Fucoidin*

MAb	Counts per minute	
	No addition	100 μg/ml fucoidin added
PH-20	74,636	109,282
PH-21	89,344	83,847
PH-22	97,294	95,504

Sperm (50% acrosome reacted) at 1×10^8/ml were incubated in medium alone or in medium containing 100 μg/ml fucoidin. After the cells were washed and subsequently incubated with excess MAbs or control (NS-1) supernatant, excess ^{125}I second antibody was added. Determinations were done in duplicate. Background counts per minute obtained in the control have been subtracted.

al. (10) that preincubation of sperm with 100 μg/ml fucoidin blocked guinea pig sperm binding to the zona of guinea pig eggs by 90% or more. Second, sperm preincubated with 100 μg/ml fucoidin were incubated with PH-20, PH-21, or PH-22. Binding of the three MAbs was not significantly inhibited by prebinding of fucoidin (Table IV). PH-20 MAb binding is somewhat increased on fucoidin-preincubated sperm. The absence of competition suggests the fucoidin and the inhibiting MAbs may be acting from distinct cell surface sites to block sperm-zona adhesion.

DISCUSSION

In experiments in which a MAb inhibits cell-cell adhesion, one explanation is that the antigen recognized by the MAb has a required function in adhesion. Alternatively, the inhibition could result from nonspecific blocking by the MAb of access to a functional molecule that neighbors the recognized antigen, or from simple prevention by the MAb of close approach of the two cells. In the case of our results, the MAbs PH-20 and PH-22 are of the same IgG subclass and bind in essentially equal amounts in the same localization on the sperm. They bind to the same antigen at epitopes close enough to block each other's binding. Since the PH-20 MAb strongly inhibits sperm-zona binding and the PH-22 MAb does not inhibit, a likely explanation is that the PH-20 antigen itself has a required function in sperm-zona binding.

We probed the relative arrangement of antigenic determinants on the PH-20 antigen by asking if the three monoclonal

antibodies would compete for binding to the antigen. We found that the PH-20 and PH-22 antibodies blocked each other's binding but had no effect on PH-21 antibody binding. One way to explain the observed inhibition of sperm-zona binding and competition among antibodies for binding to sperm is to posit that PH-21 binds an epitope relatively distant from the PH-20 and PH-22 epitopes and partially occludes the antigen's active site. PH-20 binds so as to more completely occlude the antigen's active site. PH-22 binds at an epitope close to or overlapping the PH-20 epitope but does not extend near enough the antigen's active site to affect it. Several alternative possibilities exist. They include, for example, the possibility that PH-20 and PH-22 MAbs bind at distinct epitopes (the PH-20 epitope being near the active site), but the Fc regions of the MAbs block access of the other antibody. An alternative is that the PH-20 and PH-22 MAbs might bind to overlapping epitopes at some distance from the antigen's active site and that PH-20 binding (but not PH-22 binding) might cause a conformational change in the active site.

Various immunological and biochemical approaches are being used in different laboratories with the goal of understanding sperm-egg adhesion (2, 5, 6, 8–10, 12, 16, 18, 19, 21, 23, 25, 27). In the first study to identify a sperm-egg adhesion molecule, Vacquier and Moy (25) isolated bindin from sea urchin sperm. Bindin is a 30,000-mol-wt polypeptide that exists as large aggregates in nonionic detergent (25). It therefore seems not to resemble structurally the PH-20 antigen, which apparently has a higher molecular weight (59,000–66,000) and is soluble in 1% Triton (20; Fig. 1).

Huang et al. (10) found that fucoidin blocked sperm-zona binding in guinea pig and other mammalian species. They suggested that a sperm surface macromolecule that binds fucoidin is part of the recognition system between mammalian gametes. That prebinding saturating levels of fucoidin to guinea pig sperm did not significantly reduce binding of the PH-20, PH-21, or PH-22 MAbs suggests that fucoidin and the inhibiting antibodies may be blocking sperm-zona adhesion from distinct cell surface sites.

There is strong evidence that mouse sperm surface galactosyltransferase has a required role in mouse sperm-zona binding (22, 23). In collaboration with Dr. Barry Shur (Department of Biochemistry, M. D. Anderson Hospital, Houston, TX), we found that guinea pig sperm have a surface galactosyltransferase activity that can be assayed on live cells or in detergent extracts. The relationship of this enzyme activity to the PH-20 antigen is under investigation.

Whereas it is important to learn the identity and activity of sperm molecules involved in egg binding, of equal interest is their localization on the sperm surface. Localization is presumably important in determining the geometry of sperm-zona adhesion and of sperm penetration through the zona. In this regard, our present results suggesting a role for the PH-20 antigen in guinea pig sperm-zona binding have a relationship to the findings of Huang et al. (9). These investigators reported that acrosome-intact guinea pig sperm bind loosely and transiently to the zona and only acrosome-reacted sperm bind tightly to the zona. Tight binding occurs via the inner acrosomal membrane. The PH-20 antigen has an appropriate location, the posterior head surface, to participate in binding of acrosome-intact cells to the zona (loose binding). After acrosome reaction, it migrates to the inner acrosomal membrane, the appropriate location to participate in binding of acrosome-reacted cells (tight binding). In current experiments, we are studying the possibility that antigen migration has a role in converting loose to tight binding and in establishing an appropriate orientation of sperm and egg.

We gratefully acknowledge Dr. Barry Shur for many spirited discussions on sperm-egg binding and for his collaboration in experiments on guinea pig sperm galactosyltransferase. We appreciate the advice received from Drs. Thomas Huang and Alan Fleming concerning the preparation of gametes for the sperm-zona binding assay. We thank M. Rebecca Heaton, Brian A. Patterson, and Dori Bearce for technical assistance in this project, and Joan Jannance for typing the manuscript.

This work was supported by the National Institutes of Health grant HD 16580 to D. G. Myles and American Heart Association Grant-in-Aid 82-1120 with funds contributed in part by the AHA Connecticut Affiliate. During this work, P. Primakoff held an Established Investigatorship Award from the American Heart Association.

Received for publication 3 June 1985, and in revised form 4 September 1985.

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