Lack of species-specificity in mammalian sperm chemotaxis

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Abstract

Attraction of spermatozoa by way of chemotaxis to substances secreted from the egg or its surrounding cells has been demonstrated in marine species, amphibians, and mammals. This process is species- or family-specific in marine invertebrates: a chemoattractant for one marine species is usually not recognized by another species or by a member of another family. It is not known whether this selectivity is also the rule in other phyla. Furthermore, it is not at all obvious that such selectivity would be advantageous to species with internal fertilization. Here, using a directionality-based assay for chemotaxis, we studied in vitro the chemotactic response of human and rabbit spermatozoa to human, rabbit, and bovine egg-related factors. We found that spermatozoa from each of the two sources responded similarly well to egg-related factors obtained from any of the three species examined. These results indicate lack of chemotaxis-related, species specificity between these species, suggesting that their sperm chemoattractants are common or very similar. The findings further suggest that mammals do not rely on species specificity of sperm chemotaxis for avoidance of interspecies fertilization.
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Introduction

Sperm chemotaxis—the directed movement of sperm cells up a gradient of a chemoattractant secreted from the egg or its surrounding cells—is prevalent throughout the Metazoa. It has been demonstrated in marine species with external fertilization (e.g., sea urchins and corals) (Cosson, 1990; Miller, 1985), in amphibians (Olson et al., 2001), and in mammals (Eisenbach, 1999). Mammalian sperm chemotaxis to follicular factors has been established in humans (Eisenbach and Tur-Kaspa, 1999; Ralt et al., 1991, 1994), mice (Giojalas and Rovasio, 1998; Oliveira et al., 1999), and rabbits (Fabro et al., 2002). In addition, it has been

implied in horses (Navarro et al., 1998) and pigs (Serrano et al., 2001).

An open question in mammalian reproduction is whether or not sperm chemotactic responsiveness is species-specific. Hitherto, species specificity with respect to sperm chemotaxis has only been demonstrated in marine species (Cosson, 1990; Miller, 1985, 1997). There, in some groups (e.g., hydromedusae and certain ophiuroids), the specificity is very high; in others (e.g., starfish), the specificity is at the family level and, within the family, there is no specificity. In contrast, in mollusks there appears to be no specificity at all. Likewise, in plants, a unique simple compound [e.g., fucoserratene—a linear, unsaturated alkene (1,3-*trans*-5-*cis*-octatriene)] might be a chemoattractant for various species (Maier and Müller, 1986). The situation in mammals is not known. The need for species-specificity in mammalian sperm chemotaxis is not obvious either. On the one hand,

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specificity might be desired as a means to avoid cross-species fertilization. On the other hand, specificity might not be needed in view of other known means that prevent cross-species fertilization. Thus, in addition to widespread behavioral barriers that prevent mating between individuals of different species, the female tract acts as an effective barrier against interspecies fertilization at a number of levels, from sperm transport to the ability to penetrate ova vestments (Gomendio et al., 1998 and references cited therein). Here we investigated whether or not mammalian sperm chemotaxis is species-specific.

Materials and methods

Chemicals and media

All chemicals were obtained from Sigma Chemical C. (St. Louis, MO), unless a different company was specified. The medium used (BWW) was essentially Biggers, Whitten, and Whittingham medium—95 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 20 mM sodium lactate, 5 mM glucose, and 0.25 mM sodium pyruvate, pH 7.4 (Biggers et al., 1971)—supplemented with Hepes (50 mM, pH 7.4) and BSA (fraction V powder) (3 mg/ml for human sperm, 40 mg/ml for rabbit sperm).

Animals

New Zealand white strain rabbits 8–12 months old were individually housed in standard cages in a room maintained at $20 \pm 1°C$, $60 \pm 10\%$ relative humidity, and 12 h/12 h light/dark cycles. They were used and treated in accordance with the Guide for Care and Use of Laboratory Animals (NRC, National Academy of Science, 1996).

Spermatozoa

Rabbit semen samples were collected with an artificial vagina (IMV Technologies, Cedex, France) (Bredderman et al., 1964). Human semen samples were obtained from healthy donors with normal sperm density, motility, and morphology (according to WHO guidelines of 1992) after 3 days of sexual abstinence and were allowed to liquefy for 30–60 min at room temperature. Rabbit and human spermatozoa were separated from the seminal plasma, as described earlier (Fabro et al., 2002), by the migration-sedimentation technique, which avoids the centrifugation stress (Hauser et al., 1992). Following this procedure, the sperm concentration was adjusted to 5×10^6 cells/ml. Every sperm sample was analyzed for the percentage of motile cells using a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) and a computerized sperm analysis software program (Hobson Tracking System, Sheffield, UK). The sperm suspensions were incubated under an at-

mosphere of 5% CO_2 at 37°C for 2 h (human spermatozoa) or 16 h (rabbit spermatozoa) so as to obtain sperm populations with maximal levels of capacitated spermatozoa (Chang, 1955; Cohen-Dayag et al., 1995; Giojalas et al., 2001).

Follicular factors

As sources of follicular factors we used a conditioned medium of human cumulus-oocyte complex (hCM), bovine follicular fluid (bFF), and rabbit follicular fluid (rFF). Clear conditioned medium of human cumulus-oocyte complex was obtained by the following procedure, approved by the hospital's Ethic Committee. Subsequent to oocyte retrieval from women undergoing transvaginal oocyte aspiration for in vitro fertilization, the aspirated oocyte was incubated for about 2 h in flushing medium (MediCult, Denmark). The oocyte was then removed for fertilization and the medium—hCM that may contain postovulatory egg's secretions, cumulus secretions, granulosa cells, and a tiny amount of follicular fluid in flushing medium—was used for the experiments. rFF was obtained as described previously (Fabro et al., 2002). bFF was obtained as described by Kurosaka et al. (1990) and then lyophilized. Fresh bFF was kindly provided by Dr. A. Arav (Volcani Center, Bet Dagan, Israel).

Chemotaxis assay

Chemotaxis assays were performed, as described earlier (Fabro et al., 2002), at room temperature in a Zigmond chemotaxis chamber consisting of two wells separated by a wall and closed with a coverslip (Zigmond, 1977). Unless indicated otherwise, one well (termed hereafter "the sperm well") contained spermatozoa in BWW and the other well (termed hereafter "the chemoattractant well") contained either follicular factors (bFF, rFF, or hCM) diluted in BWW or, as a control, BWW only. The movement of sperm cells on top of the partition wall, in the middle of the field between the two wells, was video-recorded for 15 min following the sealing of the chamber. The tracks made by the spermatozoa during the last 5–10 min of each recording were subsequently analyzed both semiautomatically as described earlier (Fabro et al., 2002) and fully automatically by a computerized motion analysis system (Hobson Sperm Tracking System, Sheffield, UK). Both analyses yielded similar results. Defining the X-axis as the direction of the gradient, the tracks were analyzed for chemotaxis according to the percentage of cells traveling a longer distance in the direction of the chemoattractant gradient than in a gradient-less direction, perpendicular to the former (cells with $\Delta X/|\Delta Y| > 1$) (Fabro et al., 2002).

Statistical analysis

InStat 2.01 software package (Graph Pad Software, San Diego, CA) was used for statistical calculations. The significance of the differences between the experimental mean
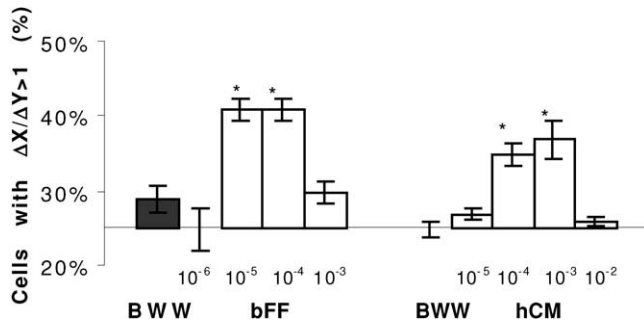
Fig. 1. Chemotactic response of rabbit spermatozoa to bFF and hCM, diluted in BWW. The chemotactic response was determined according to the percentage of cells traveling a longer distance in the direction of the gradient than in a gradient-less direction, perpendicular to the former (cells with $\Delta X/|\Delta Y| > 1$). An asterisk indicates a statistically significant difference between the column and the BWW column ($P < 0.001$ in the case of bFF, and $P < 0.02$ and < 0.005 in the case of hCM at dilutions of 10^{-4} and 10^{-3}, respectively). The results, shown with respect to the expected values in the case of random movement, are the means $\pm$ SEM of a number of experiments: in the case of bFF—12 experiments (4634 cells in total) for BWW, 3 experiments (150 cells) for the 10^{-6} dilution of bFF, 10 experiments (4110 cells) for the 10^{-5} dilution, 12 experiments (5603 cells) for the 10^{-4} dilution, and 4 experiments (200 cells) for the 10^{-3} dilution; in the case of hCM—4 experiments (927 cells) for BWW, 2 experiments (150 cells) for the 10^{-5} dilution of hCM, 4 experiments (1062 cells) for the 10^{-4} dilution, 4 experiments (1114 cells) for the 10^{-3} dilution, and 2 experiments (150 cells) for the 10^{-2} dilution.

values and the control mean values was determined in two steps. Using the χ^2 test, we first analyzed the P value of each experiment separately and then calculated the combined P value from the separate P values of all the experiments.

Results

Using a directionality-based assay, independent of the sperm's speed and pattern of movement (Fabro et al., 2002),

we examined rabbit and human spermatozoa for chemotactic cross-reactivity with follicular factors of humans, rabbits, and cows.

Chemotactic response of rabbit spermatozoa to bFF and hCM

First, as a control for random movement, we put rabbit spermatozoa in the sperm well of a Zigmond chamber and BWW in the chemoattractant well. As expected for random movement (Fabro et al., 2002), the percentages of cells with $\Delta X/|\Delta Y|$ values larger than 1 were about 25% (Fig. 1). When the chemoattractant well contained proper dilutions of bFF or hCM, the $\Delta X/|\Delta Y|$ values were significantly higher than the control, exhibiting concentration dependence typical of chemotaxis (Fig. 1). This heterospecies chemotactic response was very similar to the homospecies response observed earlier with rabbit spermatozoa and rFF [21.0 $\pm$ 1.5% in the BWW control and 24.0 $\pm$ 1.6, 46.0 $\pm$ 1.6, 40.0 $\pm$ 1.7, 30.0 $\pm$ 1.9, 23.0 $\pm$ 1.2% at rFF dilutions of 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, respectively (Fabro et al., 2002)]. Here, as in the case of the homospecies response, only a fraction of the spermatozoa [the fraction of capacitated spermatozoa (Fabro et al., 2002)] were chemotactically responsive.

Chemotactic response of human spermatozoa to bFF and rFF

To evaluate the chemotactic responsiveness of human spermatozoa to follicular factors of bovine and rabbits, we carried out the same assay, only that this time the sperm well contained human spermatozoa and the chemoattractant well contained bFF or rFF. As shown in Fig. 2, the chemotactic response of human spermatozoa to bFF and rFF was qualitatively similar to that of rabbit spermatozoa to bFF and hCM. Here, too, only a fraction of the spermatozoa [the
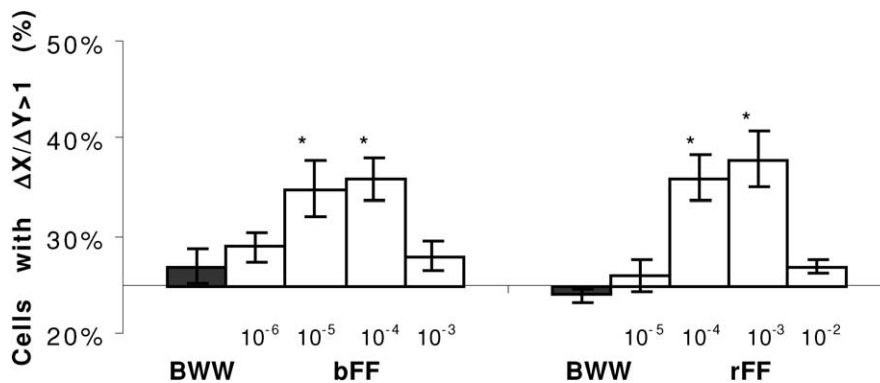

Fig. 2. Chemotactic response of human spermatozoa to bFF and rFF, diluted in BWW. The chemotactic response was determined as in Fig. 1. An asterisk indicates a statistically significant difference between the column and the BWW column ($P < 0.005$ and < 0.001 in the case of bFF at dilutions of 10^{-5} and 10^{-4}, respectively, or $P < 0.05$ and < 0.01 in the case of rFF at dilutions of 10^{-4} and 10^{-3}, respectively). The results, shown with respect to the expected values in the case of random movement, are the means $\pm$ SEM of a number of experiments: in the case of bFF—6 experiments (5017 cells in total) for BWW, 4 experiments (1447 cells) for the 10^{-6} dilution of bFF, 6 experiments (4230 cells) for the 10^{-5} dilution, 6 experiments (4700 cells) for the 10^{-4} dilution, and 4 experiments (1901 cells) for the 10^{-3} dilution; in the case of rFF—3 experiments (300 cells) for BWW as well as for each of the dilutions of rFF.

fraction of capacitated spermatozoa (Cohen-Dayag et al., 1995)] were chemotactically responsive. The fraction of responsive cells was somewhat lower than in the case of rabbit spermatozoa (8% vs. 12%, according to the percentage of cells with $\Delta X/|\Delta Y| > 1$ at bFF dilution of 10^{-5} in Figs. 1 and 2), in line with the lower level of capacitated cells in human than in rabbit spermatozoa (Cohen-Dayag et al., 1995; Fabro et al., 2002). The concentration dependence of the chemotactic response, shown in Fig. 2, was very similar to that observed when both the spermatozoa and the follicular fluid were of human (Cohen-Dayag et al., 1994; Ralt et al., 1994).

Discussion

This study demonstrates, for the first time, heterospecies chemotaxis of mammalian spermatozoa to follicular factors, suggesting that mammals do not rely on specificity of sperm chemotaxis for avoiding interspecies fertilization.

The heterospecies chemotactic response, observed in this study, was as strong as the homospecies responses observed earlier with human (Cohen-Dayag et al., 1994; Ralt et al., 1994) and rabbit (Fabro et al., 2002) spermatozoa. In sperm chemotaxis, the potency of the chemoattractant is reflected in the chemoattractant concentration that brings about a peak response. [The magnitude of the peak only reflects the fraction of chemotactic/capacitated spermatozoa (Cohen-Dayag et al., 1994, 1995).] With both human and rabbit spermatozoa, a homospecies peak response is usually obtained at $1:10^4$ or $1:10^3$ dilutions of follicular fluid (Fabro et al., 2002; Ralt et al., 1991, 1994), very similar to the dilutions needed for observing the heterospecies chemotactic responses in this study (Figs. 1 and 2). Therefore, although the identity of the mammalian sperm chemoattractant(s) is not yet known (for a review, see Eisenbach, 1999), the observed absence of species-specificity suggests that the sperm chemoattractants of mammals, at least those studied herein, are common or very similar. The observation that the threshold response of rabbit and human spermatozoa to bovine follicular fluid is lower than their threshold response to human and rabbit follicular fluids (10^{-5} vs. 10^{-4} dilution—Figs. 1 and 2) suggests that the concentration of the chemoattractant in bovine follicular fluid is higher than its concentration in human and rabbit follicular fluids, possibly reflecting the larger dimensions of this animal.

The observations made in this study may potentially facilitate the identification of the mammalian sperm chemoattractant(s). It should now be possible to purify the chemoattractant(s) from, for example, bFF, of which large quantities can be pooled together, and to assay the fractions eluted from the purification columns with spermatozoa of a species that has relatively high levels of capacitated/chemotactic cells, such as rabbit spermatozoa.

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