Reactions of antisera to hCG with living human spermatozoa

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Summary. Antisera to hCG were incubated with living human spermatozoa. Agglutination and complement-dependent immobilization of the spermatozoa occurred with the anti-hCG sera but not with control sera. The antisera also reacted with human blood cells. The anti-sperm activities were largely removed by absorbing the sera with human blood cells, although these absorptions had no apparent effect on the anti-hCG activities measured by passive haemagglutination. There was, therefore, little evidence for the presence of hCG-like molecules at the surface of living human spermatozoa.

Introduction

Asch, Fernandez & Pauerstein (1977) reported that rabbit antisera to human chorionic gonadotrophin (hCG) could be shown by immunofluorescence procedures to react with air-dried dead human spermatozoa, and it was concluded that an hCG-like substance was associated with these cells. The investigations reported in this paper were carried out to determine whether the presence of an hCG-like substance at the surface of living human spermatozoa could be detected by immunological techniques.

Materials and Methods

Materials

Sera. Rabbit antisera to hCG (5 vials bought over a period of 2½ years) were obtained from Cappel Laboratories, Cochranville, Pennsylvania and a rabbit antiserum to IgM from Miles Laboratories, Elkhart, Indiana. Normal rabbit sera were obtained from Dutch Belted rabbits (Dutchland Lab. Animals Inc., Denver, Pennsylvania). Blood was collected from the ear vein into a chilled tube and left overnight in the cold. Serum was separated from the clot by centrifugation (750 g for 12 min), re-centrifuged to remove red blood cells, heated at 56°C and frozen at -20°C in small amounts. Normal human sera were obtained from healthy male volunteers. Guinea-pig sera for use as complement were purchased from Cappel Laboratories.

Diluents. In most of the experiments phosphate-buffered saline (PBS: 9 g NaCl/l, 1.49 g Na₂HPO₄/l, pH to 7.3 with 10 N-HCl) was used. Other diluents used are specified.

HCG was obtained from Organon Inc., West Orange, New Jersey (Pregnyl: 1000 i.u./ml).

Cells. Ejaculated spermatozoa were obtained from samples provided at a male infertility clinic. Spermatozoa from 23 different men were used in the course of these experiments.

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Human blood cells were provided by a male volunteer, the blood being collected into Vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey) containing heparin as anticoagulant, or quickly transferred to such tubes from the syringe. The blood was mixed with equal volumes of Alsever’s solution (Herbert, 1978) and stored at 4°C. Rabbit blood was allowed to drip from a cut ear vein into similar heparin-containing tubes, mixed with Alsever’s solution and kept at 4°C. Formalinized sheep red cells were obtained from Cappel Laboratories.

Methods

Absorption of sera with blood cells. A mixture of blood and Alsever’s solution (2–3 ml) was diluted to 10 ml with PBS and centrifuged for 5 min at 700 g. The supernatant was discarded with the buffy coat, and the remaining blood cells were washed 6 times with PBS. After the last centrifugation the supernatant was discarded, 0.1 ml of the packed cells was added to 0.1 ml anti-hCG serum and the mixture incubated at 37°C for 1 h. The mixture was then transferred to a 400 µl microcentrifuge tube and centrifuged at 700 g for 5 min; the supernatant serum was removed and the red cells discarded. In some experiments the serum was absorbed again. Control sera were mixed with PBS instead of blood cells, then incubated and centrifuged in parallel with the absorbed sera. Absorbed sera were tested immediately or frozen (−20°C).

Assay of anti-sperm activity. The procedure was essentially that described by Hellem & Rümke (1978). Sperm samples containing approximately 100% motile cells were obtained by layering 0.5 ml PBS + 1% NHS onto 0.5 ml semen in narrow glass tubes (approximately 8 mm internal diameter), incubating at 37°C for 1 h, then carefully removing the top layer into which motile spermatozoa had moved. In some experiments the tubes were slanted to increase the surface area between spermatozoa and buffer, then returned to vertical before removing the buffer. The sperm concentration was adjusted to 15 × 10⁶/ml and 2 µl volumes were added to equal volumes of serum on Møller–Coates tissue-typing trays. The trays were incubated at 37°C for 30 min and 2 µl volumes of 1/10 diluted guinea-pig serum (complement source) were then added to each well and the trays incubated for a further 2 h at 37°C. The spermatozoa were observed with an inverted microscope and the motilities of spermatozoa in drops containing anti-hCG serum + guinea-pig serum (i.e. antiserum + complement source) were compared with those of spermatozoa in control drops containing anti-hCG serum + heated guinea-pig serum (antisemum, no added complement source) and control drops containing normal rabbit serum + guinea-pig serum (no antiserum, but complement source present). The sperm-immobilizing titres of the antisera (the most dilute solution of antiserum which caused the motility of the spermatozoa in the experimental wells to be clearly less than 50% of that in the control wells) were recorded. The sperm agglutinating activities were assayed in drops containing antiserum and heated complement. The agglutinating titre was taken as the most dilute solution causing spermatozoa to be agglutinated into tight clumps. Rabbit anti-hCG sera and rabbit anti-human IgM serum were assayed using these procedures.

Anti-human red cell activity. The procedure was similar to that used for spermatozoa. Human red cells washed 3–6 times in PBS were adjusted to approximately 7 × 10⁶/ml and added to serum on Møller–Coates trays as described above. PBS alone or PBS + 1% NHS were used as diluents.

Assay of anti-hCG activities of sera. The anti-hCG activities of absorbed and unabsorbed sera were compared by passive haemagglutination techniques, i.e. their capacities for agglutinating sheep red cells coated with an hCG preparation were compared. The procedures used were based on those described by Stavitsky (1954), Herbert (1978) and Mrs L. Cappel (personal communication).

Formalinized sheep red cells washed once in 10 ml 0.9% (w/v) NaCl, and twice in PBS, pH 7.2 (Stavitsky, 1954), were packed by centrifuging the suspension at 700 g for 5 min. Cells were resuspended to 2.5% ((volume of packed cells/volume of red cell suspension) × 100 = 2.5)
in PBS, pH 7.2. Then 2 ml of this suspension were mixed with an equal volume of 1/20 000 tannic acid (stock solution, 1/100 in 0.9% NaCl diluted to 1/20 000 with PBS, pH 7.2), and left for 1 h at room temperature with occasional shaking. The cells were then centrifuged, washed once in PBS, pH 7.2, re-suspended in about 2 ml PBS, pH 6.4 (Stravitsky, 1954), 0.6 ml hCG preparation was added, and the solution made up to 4 ml with PBS, pH 6.4, so that the final concentration of red cells was 1.25%. The suspension was left at room temperature for 4 h, shaking occasionally, then centrifuged at 700 g for 5 min before washing the red cells 3 times in 1% NRS in saline (Stravitsky, 1954). These hCG-coated red cells (0.1 ml volumes) were added to equal volumes of anti-hCG serum diluted in saline + 1% NRS or PBS + 1% NHS in round-bottomed plastic tubes (internal diameter 13 mm). The degree of agglutination was graded according to the criteria described by Stavitsky (1954). Tubes containing diluent alone and formalinized cells which had been through a procedure similar to that described above, but without the addition of hCG, provided negative controls.

Results

The rabbit anti-hCG sera showed clear reactions with living human spermatozoa, causing tail-to-tail agglutination and complement-dependent sperm immobilization. The modal sperm immobilizing titre was 1/40 (most frequently obtained value in 21 experiments). Agglutination titres ranged from 1/10 to 1/80 with the majority (20/23 determinations) being ≥1/20. Duplicate assays carried out on the same day with the same spermatozoa gave either identical titres or titres differing by only one dilution step, but the range of values obtained in experiments carried out with different samples of sera and spermatozoa were greater (<1/10–1/80). Most of this variability apparently resulted from variations in sensitivity of the different sperm suspensions used. The anti-sperm activities observed with the anti-hCG sera were not observed with rabbit anti-human IgM serum at the dilutions tested (most concentrated tested = 1/5 dilution).

The rabbit anti-hCG sera also reacted with human blood cells and the haemolytic titres of the antisera (i.e. the dilutions of antisera which clearly lysed more than 50% of the red cells) ranged from 1/20 to 1/80 in experiments carried out with different samples of antisera and different lots of red cells. The modal haemolytic titre was 1/40 (most frequently obtained value in 7 experiments). The anti-hCG sera apparently caused some agglutination of red cells in these tests, and also in tubes when 0.1 ml volumes of diluted antisera were mixed with equal volumes of 1% solutions of red cells, but no attempt was made to titrate this activity.

Absorption of the anti-hCG sera with human red cells always caused clear reductions in the anti-sperm titres of the sera, i.e. reduction of two or more dilution steps (10/10 experiments in which spermatozoa immobilizing activities were assayed and 9/9 experiments in which sperm-agglutinating activities were assayed), while absorptions with rabbit red cells did not (0/6 experiments in which sperm-immobilizing activities were assayed and 0/6 experiments in which sperm-agglutinating activities were assayed). The differences between the two types of absorption were significant (P < 0.0002, Fisher’s Exact Test). The results of experiments in which fairly concentrated dilutions of absorbed anti-hCG sera were tested (most concentrated tested ≤1/1.25) are summarized in Table 1. The anti-sperm activities of the sera were very largely removed by absorption with human blood cells, but not by rabbit blood cells.

Absorptions of the sera with blood cells did not have comparable effects on the anti-hCG activities measured. While the sperm agglutinating titres were always reduced by two or more dilution steps after absorption with human red cells (9/9 experiments, see above) the anti-hCG titres were not (0/3 experiments). In these 3 experiments, in each of which the titrations were carried out in duplicate or triplicate, the unabsorbed sera gave anti-hCG titres in the range 1/100–1/200 (6/7 titres = 1/200) while sera which had been absorbed with human red cells or
Table 1. Absorption of anti-human sperm activity from anti-hCG serum by red cells

<table>
<thead>
<tr>
<th>Type of absorption</th>
<th>Range of titres of unabsorbed sera in different experiments</th>
<th>Range of titres of absorbed sera in different experiments</th>
<th>% activity detected after absorption (±s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbed (1 ×) with human red blood cells</td>
<td>1/10–1/80</td>
<td>&lt;1/1·25–1/2·5</td>
<td>3·1 ± 3·1 (4)</td>
</tr>
<tr>
<td>Absorbed (1 ×) with rabbit red blood cells</td>
<td>1/10–1/80</td>
<td>1/10–1/80</td>
<td>100 (2)</td>
</tr>
<tr>
<td>Absorbed (2 ×) with human red blood cells</td>
<td>1/10–1/40</td>
<td>&lt;1/1·25–1/2·5</td>
<td>2·0 ± 0·4 (6)</td>
</tr>
<tr>
<td>Absorbed (2 ×) with rabbit red blood cells</td>
<td>1/10–1/40</td>
<td>1/10–1/40</td>
<td>100 (4)</td>
</tr>
<tr>
<td>Absorbed (1 ×) with human red blood cells</td>
<td>1/10–1/40</td>
<td>≤1/1·25</td>
<td>6·3 ± 6·3 (2)</td>
</tr>
<tr>
<td>Absorbed (1 ×) with rabbit red blood cells</td>
<td>1/10–1/40</td>
<td>1/10–1/80</td>
<td>≥100 (2)</td>
</tr>
<tr>
<td>Absorbed (2 ×) with human red blood cells</td>
<td>1/10–1/80</td>
<td>&lt;1/1·25–1/2·5</td>
<td>1·6 ± 0·7 (5)</td>
</tr>
<tr>
<td>Absorbed (2 ×) with rabbit red blood cells</td>
<td>1/40–1/80</td>
<td>1/40–1/80</td>
<td>87·5 ± 12·5 (4)</td>
</tr>
</tbody>
</table>

In each experiment the anti-sperm activities of control unabsorbed sera were compared with those of absorbed sera and the % activity detected after absorption calculated from the formula: (titer after absorption/titer before absorption) × 100. The means of these values are given for the number of experiments indicated in parentheses.

rabbit red cells gave anti-hCG titres of 1/100 (all replicates). These differences between the effects of absorption on anti-sperm activity and the effects on hCG activity were statistically significant (P < 0·005, Fisher's Exact Test). The differences between the effects of absorption with human red cells and the effects of absorption with rabbit red cells which were observed when the anti-sperm activities were compared (see Table 1) were not observed when the anti-hCG activities were compared (see results summarized above).

Discussion

The experiments reported have provided no substantial evidence that an hCG-like molecule was detectable at the surface of living human spermatozoa. Anti-hCG sera did show reactions with living human spermatozoa which were not shown by normal rabbit sera or rabbit anti-human IgM serum but these anti-sperm activities were largely removed by absorption with human red cells (Table 1) without, apparently, any comparable effects on the anti-hCG activities of the sera. The anti-sperm activities of the anti-hCG sera found in these experiments could probably be explained by immunological cross-reactivity, i.e. by the presence on the surface of human spermatozoa and somatic cells of antigenic sites similar to some found on the hCG molecule. There have been reports (e.g. Braunstein, Kandar, Rasor, Swaminathan & Wade, 1979; Yoshimoto, Wolfsen, Hirose & Odell, 1979) of hCG-like substances in a variety of normal human tissues but the results reported in this paper, taken in isolation, could not be taken to provide evidence that the molecules on living human spermatozoa and red cells with which these rabbit anti-hCG sera reacted were sufficiently similar to hCG to justify the term hCG-like.

The present observations are different from those of Asch et al. (1977), who found that anti-hCG serum reacted with spermatozoa but not red blood cells. However, Asch et al. (1977) examined dead spermatozoa and the antigens exposed in living and dead cells may be different. Our results do, however, emphasize that there is so far little evidence that hCG-like molecules
are accessible on living human spermatozoa and are of some relevance when considering the possible biological role of an association between an hCG-like substance and human spermatozoa (Asch et al., 1977; Asch, Fernandez, Siler-Khodr & Pauerstein, 1979).

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References


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