Optimized assay for antisperm cell-mediated immunity

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Previous studies on antisperm cell-mediated immunity (CMI) have been confounded by the presence of immunogenic leukocytes in sperm antigen preparations. In this study we isolated pure populations of viable spermatozoa on discontinuous Percoll gradients, and utilized sonicated and cavitated extracts, as well as live motile spermatozoa, to measure cellular immunity to spermatozoa in vasectomized men, men with proven fertility, infertile women, fertile women and umbilical cord blood. Using a thymidine incorporation assay to assess lymphocyte proliferation, nine out of 13 (69%) vasectomized men and five out of 10 (50%) fertile men responded to sperm extracts. Lymphocyte proliferation to sperm extracts was also observed in both infertile and fertile women (27 and 50% respectively). In addition, viable sperm preparations promoted lymphocyte responses in five out of eight (63%) fertile women, seven out of 11 (63%) healthy men and four out of 11 (45%) cord blood specimens. Furthermore, four out of 11 (36%) healthy normal men responded to autologous spermatozoa. No relationship between serum antisperm antibodies, as measured with the Immunobead test, and sperm CMI was observed in any group. This study provides evidence that lymphocytes from fertile as well as infertile men and women and sperm-naive newborn infants proliferate when exposed to viable spermatozoa or sperm extracts. Thus the lymphocyte proliferation assay does not appear to be useful in the diagnosis of immunological infertility, but immunological recognition of spermatozoa may be a common feature that could have a role in fertility.

Key words: cellular immunity/infertility/lymphocyte proliferation/spermatozoa/vasectomy

Introduction

Antisperm antibodies have been detected in the sera of infertile couples (Isojima et al., 1968; Haas et al., 1980), and their presence is reported to correlate with poor fertility rates (Gupta et al., 1975). However, antisperm antibodies may also occur in proven fertile men (Bronson et al., 1984; Shai and Naot, 1992; Snow and Ball, 1992). Thus the clinical significance and predictive value of antisperm antibodies in infertility remains unclear (London et al., 1984; Collins et al., 1993).

The role of antisperm cell-mediated immunity (CMI) in reproductive processes also remains enigmatic. Antisperm CMI responses have been documented in all the following groups of individuals: infertile men and women (Tyler et al., 1967); fertile and infertile women (Mettler and Schirwani, 1975; McShane et al., 1985; Schroeder et al., 1990); virgin women (Mallman et al., 1991); oligozoospermic men (El-Alfi and Bassili, 1970); and men with andrological disorders (Polidori et al., 1980).

CMI studies have been confounded technically by the use of different assay techniques and protocols, different patient groups and sperm preparations contaminated by white blood cells (WBC). The latter variable is of particular concern when assessing the results obtained in previous studies. Although not expressed on spermatozoa (Anderson et al., 1982), major histocompatibility complex (MHC) class I antigens are found on almost all other cells. In addition, MHC class II antigens are expressed on monocytes, macrophages, dendritic cells, B cells and activated T cells.

Even small numbers of these cells can trigger a mixed leukocyte reaction (MLR). Normal human ejaculates contain a median of 1.7×10^6 leukocytes (Wolff and Anderson, 1988), and specimens with counts >10^6 WBC/ml are relatively common (Wolff et al., 1990). Previous studies utilizing spermatozoa in lymphocyte transformation assays have produced conflicting results (Therstrup-Pedersen et al., 1976; Misko et al., 1978). It is therefore probable that, at least in some previous studies, positive results in sperm CMI assays may have been the result of an MLR and not of reactivity against sperm antigens.

Developing a test to evaluate antisperm CMI may be clinically useful in investigating infertility in both sexes, and may ultimately be used to screen for a number of immunological disorders in reproduction. The purpose of this study was to determine whether leukocyte-free preparations of human spermatozoa stimulate lymphocyte proliferation in fertile and/or infertile subjects, with the ultimate aim of providing an optimized antisperm CMI assay for clinical use.

Materials and methods

Study groups

Peripheral blood was obtained from the following groups of individuals by antecubital venepuncture. Group I comprised 12 vasectomized men (mean post-operation time 10.7 ± 7.2 years, range 2–21) and one man with an earlier episode of testicular torsion (5 years). The mean age of this group was 45.0 ± 9.3 years (range 35–62),...
and 12 of the 13 had fathered a child. Group 2 contained 11 non-
vasectomized proven fertile men (mean age 33.9 ± 5.7 years, range
32–51). Group 3 included 10 non-vasectomized healthy men of
unproved fertility (mean age 31.0 ± 6.2 years, range 20–42). Group
4 comprised 11 infertile women (mean age 35.5 ± 4.3 years, range
27–41) randomly selected from the infertility service of the Brigham
and Women’s Hospital (Harvard Medical School, Boston, MA, USA)
with the following diagnoses: laparoscopic diagnosis of endometriosis
(n = 4), polycystic ovarian syndrome (n = 2), fibroids and pelvic
adhesions (n = 2), and infertility of unknown origin (n = 3). Group 5
included 10 women of proven fertility (mean age 35.5 ± 5.8 years,
range 29–48). Group 6 comprised umbilical cord blood from 11
newborn babies (six female, five male) obtained immediately after
delivery at the Brigham and Women’s Hospital.

Antisperm antibody assay

All of the vasectomized and control men were tested for serum
antisperm antibodies by the Immunobead Test (Total Immunoglobulins
IBT; Bio-Rad, Richmond, CA, USA) (Bronson et al., 1983).

Semen samples

Fresh ejaculates from non-leukocytospermic donors (World Health
Organization, 1992) were obtained by masturbation into sterile plastic
containers after a minimum sexual abstinence period of at least 48 h.
Samples were liquefied for 30 min at room temperature and used
within 2 h.

Separation of WBC from spermatozoa

Sewin-up technique (modified from Arny and Quagliarello, 1987)
Semen was mixed with 3 ml Biggers–Whitten–Whittingham (BWW)
medium/2% human serum albumin (HSA) in a 15 ml round-bottomed
tube (Falcon; Becton Dickinson, Mountain View, CA, USA) and
centrifuged for 10 min at 400 g. The supernatant was discarded and
the pellet resuspended in 2 ml BWW medium/HSA. The resultant
supernatant was centrifuged for 10 min at 400 g. The supernatant was
discarded and 1 ml BWW medium/HSA gently layered over the
pellet. The tube was placed at an angle and incubated for 1 h at 37°C
in a humidified CO2 incubator. The supernatant and pellet were used
for leukocyte analysis.

Ficoll technique

One-quarter of each sample was diluted 1:1 with BWW medium/
HSA, gently layered on 3 ml Ficoll-Paque (Pharmacia, Uppsala,
Sweden) in a 15 ml conical tube (Falcon; Becton Dickinson) and
centrifuged for 30 min at 1500 g. The cells at the Ficoll–semenal
plasma interface and at the bottom of the tube were used for
leukocyte analyses.

Discontinuous Percoll gradient

This was adapted from a method by Berger et al. (1985). A
discontinuous Percoll (99% pure; Pharmacia) gradient was made by
layering 2 ml 47% Percoll onto 2 ml 90% Percoll in a 15 ml tube.
Semen was diluted 1:1 in Ham’s F-10 medium (Gibco, Grand Island,
NY, USA), gently layered over the 47% Percoll layer and centrifuged
at 600 g for 30 min. Cells at the 47% and 90% Percoll interfaces as
well as in the pellet were saved for leukocyte analyses.

Detection of WBC

In the preliminary ‘contamination’ experiments, one-quarter of eachaw specimen and all cellular fractions obtained from the various
separation techniques were washed twice in phosphate-buffered saline
(PBS), resuspended in a minimum volume of PBS (concentration
<50×10⁶/ml), and 5 µl drops applied to eight spot slides (Roboz
Surgical, Washington, DC, USA). After air drying and a 10 min
acetone fixation, slides were stored at −70°C until use. Slides were
stained with an anti-CD45 monoclonal antibody (HLe-1, Dako- LC;
Dako Corporation, Santa Barbara, CA, USA), and a standard alkaline
phosphatase/anti-alkaline phosphatase technique was used for the
detection of leukocytes (Politch et al., 1993). Leukocyte concentra-
tions were determined by referring to total ‘round cell’ count in the
original sample and the various fractions of the separation procedures,
as determined microscopically by a haemocytometer.

To ensure the purity of each preparation used in the CMI assays,
individual semen samples were processed separately and pellets
examined by phase microscopy in a Makler chamber (Zygotek
Systems Inc., Springfield, MA, USA). Samples with more than one
leukocyte per 100 spermatozoa were discarded.

Antigen preparations

Sonicated sperm antigens (SSA)
Percoll sperm pellets obtained from multiple donors were pooled,
washed twice in Ham’s F-10 medium and adjusted to 50×10⁶/ml.
Whole spermatozoa were disrupted with four sonic bursts of 20 s
each on ice (using a Bruanson 220 sonifier; Braunson Cleaning
Equipment Company, Shetton, CT, USA; at 20% maximum output),
centrifuged at 10 000 g at 4°C for 10 min, and the supernatants stored
at −70°C.

Cavitated sperm antigens (CSA)
This procedure was modified from Mack et al. (1986). Fresh Percoll
sperm pellets were pooled from multiple donors, adjusted to a
concentration of 50×10⁶ spermatozoa/ml and placed in the chamber
of a clean cell disrupter bomb (Parr Instruments, Moline, IL, USA).
The chamber was filled with nitrogen to 600 psi for 10 min. Then
the pressure was suddenly released, and the cavitated sperm suspension
poured into a 15 ml Falcon tube and centrifuged at 6000 g for 10
min at 4°C. The resultant supernatant was centrifuged at 20 000 g
for 20 min at 4°C to pellet cellular organelles and small debris. Then
the supernatant was ultracentrifuged at 100 000 g for 60 min at 4°C
to pellet cell membranes. The membranes were resuspended in 10 mM
Tris–Cl (0.5 mM MgCl2, 0.6 NaCl, pH 7.6). The protein concentration
was determined using the bicinchoninic acid (BCA) reagent kit
(Pierce, Rockford, IL, USA). Cavitated samples were aliquoted and
stored at −70°C until use.

Live spermatozoa
Spermatozoa from the Percoll pellet were washed three times in
Ham’s F-10 medium and resuspended in Ham’s F-10 medium plus
0.3% AB+ human serum (Gibco).

Red blood cell (RBC) membranes
RBC were recovered from peripheral blood after the removal of
WBC by Ficoll-Hypaque (Pharmacia) gradient centrifugation (30 min
at 600 g) and the removal of the buffy coat containing neutrophils
by pipette. PBS-washed RBC were incubated with a hypotonic lysis
solution (10 mM Tris, pH 7.6) and the membranes were washed three
times at 500 g for 10 min to remove haemoglobin. The pellet was
washed three times (Gibco) and sonicated as described above.

Other antigens and mitogens
Phytohaemagglutinin (Sigma, St Louis, MO, USA; 1 µg/ml) and
tetanus toxoid (TT; Massachusetts Public Laboratories, Boston, MA,
USA; 0.1, 1 Lf/ml) were also used as positive controls for antigen
responsiveness.

Lymphocyte proliferation assay
Peripheral blood mononuclear cells were isolated from heparinized
blood by Ficoll-Hypaque gradient centrifugation. Cells were washed
twice with Hank’s medium (Gibco) and resuspended at 1×10⁶/ml
Cellular immunity to spermatozoa

with RPMI 1640 supplemented with 0.3 mM l-glutamine, antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin; Sigma) and 10% AB+ human serum to a concentration of 1×10^3/ml. This medium was used throughout the assay. Aliquots of 100 ml were added to 96-well round-bottomed plates (Corning, Corning, NY, USA). The various antigens were added at different concentrations in triplicate wells to a final volume of 200 µl. After 5 days (6 days for live sperm experiments), cultured cells were pulsed with 0.5 µCi/well [³H]thymidine (New England Nuclear, Boston, MA, USA) and harvested onto filter discs 16–18 h later using a cell harvester (PHD Instruments, Watertown, MA, USA). The incorporation of [³H]thymidine was measured by liquid scintillation counting in a Beta-Counter (Tm Analytic, Brandon, FL, USA). The stimulation index (SI) was calculated (SI = mean c.p.m. with antigen/mean c.p.m. without antigen). An SI value >2 was considered to be evidence of a specific lymphocyte proliferation in response to antigen (Therstrup-Pedersen et al., 1976).

Statistical analysis

Statistical analyses were performed using Fisher’s exact test and the Mann–Whitney U-test on a Macintosh II ci computer (Apple Computer Inc., Cupertino, CA, USA) with the Statview 4.0 statistical program (Abacus Concepts Inc., Berkeley, CA, USA).

Results

A comparison of sperm purification techniques

In preliminary experiments, semen samples were divided into four aliquots and the different methods (swim-up, Ficoll and Percoll) of purifying spermatozoa were compared for their ability to remove seminal leukocytes (Figure 1). Recovery of WBC from the different techniques was not 100% because of a significant loss during the wash procedures. The Percoll technique yielded spermatozoa with the least leukocyte contamination of the three separation methods. After separating semen cells with this technique, the majority of round cells (leukocyte and immature germ cells), along with some immobile spermatozoa, were found in the upper band. Smaller numbers of round cells were seen in the lower band, along with immotile spermatozoa. The pellet contained motile spermatozoa, and no round cells were detected. Thus the Percoll method was used to prepare leukocyte-free spermatozoa for CMI assays.

CMI responses to SSA and CSA

SSA were tested at concentrations between 0.06 and 64.00 µg/ml and CSA at concentrations between 0.02 and 30.00 µg/ml. Previously these ranges of doses had been shown to give positive proliferative responses in a number of vasectomized men who were used to screen the antigen (Figure 2). An SI value >2 was considered to be positive. Vasectomized (group 1) and non-vasectomized (group 3) men were tested on at least three separate occasions; individuals were considered to be responders if they were positive in all, or the majority of, assays. Because blood samples from women (groups 4 and 5) were obtained in the clinic, these individuals were tested only once, as were cord blood samples (group 6). Each individual was tested in a proliferation assay on at least three separate occasions, and the highest SI value obtained for an individual was used for the statistical analysis. Data are summarized in Table I.

Eight of the 13 vasectomized men (62%; group 1) and four of the 10 (40%) non-vasectomized (group 3) men responded to SSA (median SI values 2.8 for group 1 and 3.7 for group 3). Four of the 10 (40%) vasectomized (group 1) men and four of the 10 (40%) non-vasectomized (group 3) men responded to CSA (median SI values 3.7 for group 1 and 4.7 for group 3). Overall, the number of vasectomized (group 1) men responding to at least one of the antigens was 69%, and the number of fertile men responding was 50% (group 2). Differences between the two groups with either antigen were not statistically significant.

One of the 11 infertile women (group 4) responded to SSA (median SI 2.4), and two of the 11 infertile women responded to CSA (median SI 4.5). In group 5, positive SI values to both SSA (median SI 3.5) and CSA (median 12.5) were noted in four (40%) fertile women. Although the SI values for the fertile women appeared to be markedly higher than those for the infertile women (group 4), the difference was not statistically significant with this small sample size.
Table I. Proliferation of peripheral blood mononuclear cells in response to sperm antigens

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Positive SI (&gt;2) to SSA</th>
<th>Positive SI (&gt;2) to CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasectomized men (n = 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>8/13 (61.5)</td>
<td>4/10 (40.0)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.9 ± 2.8</td>
<td>6.1 ± 5.5</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.4–10.8</td>
<td>2.8–14.4</td>
</tr>
<tr>
<td>Median</td>
<td>2.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Fertile men (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>4/10 (40.0)</td>
<td>4/10 (40.0)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.1 ± 2.0</td>
<td>6.8 ± 6.2</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.2–6.9</td>
<td>2.3–15.7</td>
</tr>
<tr>
<td>Median</td>
<td>3.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Infertile women (n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>1/11 (9.1)</td>
<td>2/11 (18.1)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.4</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Range of positives</td>
<td>NA</td>
<td>4.4–4.7</td>
</tr>
<tr>
<td>Median</td>
<td>NA</td>
<td>4.5</td>
</tr>
<tr>
<td>Fertile women (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>4/10 (40.0)</td>
<td>4/10 (40.0)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.2 ± 12.4</td>
<td>14.5 ± 13.5</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.1–27.9</td>
<td>2.6–30.5</td>
</tr>
<tr>
<td>Median</td>
<td>3.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Table II. Proliferation of peripheral blood mononuclear cells in response to motile spermatozoa

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Positive SI (&gt;2) to live spermatozoa (0.5–30 × 10⁵/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord white blood cells (n = 11)</td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>5/11 (45.4)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.6 ± 1.6</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.1–6.3</td>
</tr>
<tr>
<td>Median</td>
<td>3.4</td>
</tr>
<tr>
<td>Fertile women (n = 8)</td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>5/8 (62.5)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.0–4.9</td>
</tr>
<tr>
<td>Median</td>
<td>2.4</td>
</tr>
<tr>
<td>Control men (n = 11) (stimulated with heterologous spermatozoa)</td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>7/11 (63.6)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.0–3.5</td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
</tr>
<tr>
<td>Control men (n = 11) (stimulated with autologous spermatozoa)</td>
<td></td>
</tr>
<tr>
<td>No. positive (%)</td>
<td>4/11 (36.3)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.7 ± 1.8</td>
</tr>
<tr>
<td>Range of positives</td>
<td>2.3–6.4</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
</tr>
</tbody>
</table>

SI = stimulation index.

**TT** was also used as a positive control for recall antigen. No significant differences were noted in the number of positive responders in the different groups. Eight of the 11 men in group 1 (median SI 10.1), six of the 11 fertile men in group 2 (median SI 7.6), all of the infertile women in group 4 (median SI 7.1) and nine of the 10 fertile women in group 5 (median SI 9.5) responded to TT antigen. None of the individuals responded to RBC membranes.

**CMI responses to live spermatozoa**

CMI responses to live autologous and heterologous spermatozoa were investigated in fertile men (group 2). Responses to heterologous spermatozoa were also studied in fertile women (group 5) and in freshly obtained cord blood specimens (group 6). Sperm concentrations ranged from 0.5 to 50.0 × 10⁵/ml. Data from these viable sperm stimulation studies are presented in Table II.

Seven of 11 non-vasectomized fertile men (group 2; 64%) responded to heterologous spermatozoa (median SI 2.7), and four of these men (36%) also responded to autologous spermatozoa (median SI 3.0). Five of the eight fertile women (group 5; 65%) and five of the 11 (45%) cord blood samples (group 6) also had an SI >2 (median SI 2.4 and 3.4 respectively). As with the previous set of experiments, similar numbers of healthy men and women responded to TT. None of the cord blood samples registered a response to TT.

**Lack of a relationship between antisperm antibodies and CMI to spermatozoa**

Of the 13 vasectomized men tested for antisperm antibodies, one was repeatedly positive and three were borderline positive. None of the control men in groups 2 and 3 had antisperm antibodies. There was no correlation between antisperm CMI responses and antisperm antibodies.

**Discussion**

A substantial body of literature documents cell-mediated responses to whole spermatozoa and to sperm antigens in various groups of individuals (El-Alfi and Bassili, 1970; Mettler and Schirwani, 1975; Naz and Metha, 1989; Mallmann et al., 1991; Dimitrov et al., 1992). However, little attention has been paid to the possibility that contaminating WBC in semen can cause false-positive responses. Even small numbers of leukocytes can induce an MLR. The aim of this study was to establish a technique for removing leukocytes from spermatozoa, and to use pure spermatozoa and sperm extracts in tests of CMI in various male and female populations.

The commonly used discontinuous Percoll gradient method effectively removed leukocytes to produce a pure population of viable spermatozoa which subsequently could be used for CMI studies. Then we compared the antigenic activity of live spermatozoa and sperm antigen preparation. A standard lymphocyte proliferation assay was used to detect antisperm CMI responses. This assay is deemed to be sensitive and can be used in a clinical laboratory environment. Initially we tested the sperm antigen preparations on blood from vasectomized men (group 1), because this group had an increased incidence of antisperm immunity because of exposure to sperm antigens following the surgical procedure (Jenkins et al., 1979; Nagarkatti and Rao, 1976). In the vasectomized group (group 1), 69% of men registered a positive SI to SSA or CSA. There was no apparent relationship between CMI responsiveness and the presence of antisperm antibodies, as detected by an immunobead assay. However, an unexpected outcome of this study was a high response rate in non-vasectomized men (groups 2 and 3). Live heterologous spermatozoa were also
efficient at activating lymphocytes from 64% of the non-vasectomized (groups 2 and 3) men, although we observed an intra-individual variation in response to heterologous spermatozoa. Stimulation with autologous spermatozoa was also observed in 36% of these men. It is possible that the increased incidence and variability in response to heterologous spermatozoa reflect the presence of polymorphic sperm antigens such as those observed in mice (Xu and Anderson, 1987).

Historically it has been hypothesized that the blood–testis barrier prevents developing spermatozoa and immunocytes from coming into contact. Perturbation of the blood–testis barrier has been shown to induce experimental autoimmune orchitis in guinea pigs (Brown and Glynn, 1969) and in a murine model (Mahi-Brown and Tung, 1989). More recently, it has been hypothesized that this barrier may not be complete and that regulatory or suppressor CD8+ cells might also play an active down-regulating role (Ritchie et al., 1984) along with other immunoregulatory mechanisms (Hurtenbach et al., 1984; Anderson and Hill, 1989) in preventing inappropriate immunological reactions to spermatozoa. This is supported by data indicating that germ cells have been observed outside the blood–testis barrier (Yule et al., 1988).

Recent studies have also shown that T cell proliferative responses to other self-antigens, such as myelin basic protein, are present in normal subjects as well as in multiple sclerosis patients, although at a much lower frequency (Ota et al., 1990; Wucherpfennig et al., 1991). Thus, T cells responding to self-antigen in an appropriate and regulated manner have been hypothesized to be part of an active and required tolerogenic network (Cohen, 1992). The possibility that some antigens present on spermatozoa are shared with other cell types and are thus tolerized centrally is supported by the observation that cord blood cells recognize spermatozoa and sperm products.

Lymphocytes from 27% of the infertile group of women (group 4) and 50% of the fertile women (group 5) also proliferated after exposure to sperm extracts (difference not statistically significant). The stimulation indices for the two sperm antigen preparations were considerably higher in the fertile women than in the infertile women (median SI values of 3.5 and 12.5 for the cavitated versus 2.4 and 4.5 for the sonicated extracts respectively). When live spermatozoa were used as an antigen source, five out of eight (62%) of the fertile women (group 5) responded.

The immune response of a female to spermatozoa is, theoretically, the same as to any other foreign antigen at a mucosal surface. However, within the context of sexual intercourse, there are a number of additional parameters that must be considered in assessing the outcome of ‘immunization’. Firstly, spermatozoa do not express MHC antigens (Anderson et al., 1982), although they express other polymorphic and tissue-specific antigens which are immunogenic. Secondly, the immunogen (spermatozoa) is carried in seminal plasma, which is a complex fluid containing highly immunosuppressive molecules (Alexander and Anderson, 1987) such as the prosta-glandins of the E series (Quayle et al., 1989). Thirdly, seminal plasma also contains factors that cause leukocytosis (Anderson and Alexander, 1979; Pandya and Cohen, 1985), and sperm phagocytosis is often observed in the female reproductive tract after coitus (Thompson et al., 1991; Tomlinson et al., 1992). We hypothesize that responses to spermatozoa are normal, but in this environment result in the induction of suppressor-like T cells and may be related to tolerogenic mechanisms similar to those of the gastrointestinal tract (Mowat, 1987). Preliminary analyses made in our laboratory at the cytokine level indicate that there may be an activation of inappropriate T cell subsets in infertile women. Thus, there may be fundamental qualitative differences in cellular immune responses to spermatozoa in the infertile women which are associated with infertility or impaired reproduction. As an example of an inappropriate immune response during pregnancy, the activation of Th2 cells may play an important role in pregnancy maintenance, whereas the activation of Th1 cells may cause abortion (Hill et al., 1995). Th1 cytokines have detrimental effects on spermatozoa (Hill et al., 1987a), mouse embryo development (Hill et al., 1987b) and implantation (Haimovici and Anderson, 1993), and may mediate reproductive failure.

In conclusion, the use of highly purified sperm preparations has allowed us to demonstrate the presence of an antisperm response in fertile as well as infertile individuals. We are presently investigating cytokine profiles in response to sperm antigens in these patient populations.

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