Suppression of Epididymal Sperm Antigenicity in the Rabbit by Uteroglobin and Transglutaminase in vitro

Abstract. *There is evidence that the mammalian female genital tract is capable of responding immunologically when challenged with alloantigens. The antigenic properties of male gametes have been well delineated. However, it is only rarely that a female mammal ever responds immunologically to the male gametic antigens as a result of coitus. When a proposed mechanism of suppression of antigenicity of epididymal spermatozoa was tested experimentally, the results indicated that two proteins (uteroglobin and transglutaminase) present in the prostate may be responsible for suppressing sperm antigenicity in the rabbit.*

The mammalian female, during coitus, normally receives in her reproductive tract semen from a genetically dissimilar male. Antigenic properties of spermatozoa have been well established in experimental animals (*1*) as well as in man (*2*). It has also been demonstrated that the female reproductive tract is not an immunologically privileged organ (*3*). Nevertheless, ejaculated spermatozoa normally do not immunize the females as a consequence of coitus. Several studies suggest that prostatic secretions have immunosuppressive factors (*4*). However, the actual mechanism of this nonimmunogenicity of ejaculated spermatozoa in the female genital tract is not well understood. Here, we describe some experiments conducted with rabbit cells in vitro that suggest that at least two components of prostatic fluid, namely, uteroglobin (UG) and transglutaminase (TG), may be involved in suppressing the antigenicity of epididymal spermatozoa.

For our experiments we used mixed cultures of splenocytes and epididymal spermatozoa. The splenocytes were used as responder cells which, when stimulated with the antigens of the epididymal spermatozoa (stimulator cells), incorporated [^{3}H]thymidine. The epididymal spermatozoa were treated in various ways to ascertain the effects of UG and TG in masking the sperm antigens (see Table 1).

Splenocytes were prepared from adult male and female New Zealand rabbits (3 to 3.5 kg) by a modified standard method (*5*). Briefly, spermatozoa were isolated by dissecting the epididymis from one animal in 3 ml of Hanks balanced salt solution (HBSS). The sperm suspension was centrifuged at 2000 rev/min in a Sorvall RC-5B centrifuge and the pellet was washed twice with HBSS before a cell count was made by using a hemocytometer. We mixed 5×10^6 splenocytes with 5×10^5 spermatozoa. Chromosome medium 1A without phytohemagglutinin (PHA) (Gibco) was used to culture the splenocyte-sperm mixture. Each culture was done in triplicate. The viability of the splenocytes was assessed by the trypan blue dye exclusion test as well as by PHA stimulation and [^{3}H]thymidine incorporation. After 4 days of culture at 37°C in a CO_2 incubator, [^{3}H]thymidine (specific activity, 1600 mCi/mM, Schwarz/Mann) at a final concentration of 1 μCi/ml was added and the culture was incubated for another 24 hours. The cultures were then spun at 3000 rev/min for 10 minutes in a Beckman TJ-7 centrifuge and the cell pellets were washed three times with HBSS. The cell pellets were then filtered in a Millipore filter type HA with a pore size of 0.45 μm under vacuum. The filtrate was washed one time with 50 percent cold trichloroacetic acid (TCA) and five times with 5 percent cold TCA. The filters were air-dried and 5 ml of Aquasol was added in a scintillation vial. The samples were counted in a Beckman LS-9000 scintillation counter. The results were expressed as counts per minute ± the standard error of the mean.

Splenocytes from both autologous and heterologous males and females, in a mixed culture with epididymal spermatozoa, incorporated [^{3}H]thymidine but to a much lesser degree compared to the PHA-stimulated controls. Autologous splenocytes incorporated [^{3}H]thymidine slightly less than the heterologous male or female cells (Table 1). However, lymphocyte stimulation was dramatically suppressed when the epididymal sperm had been treated with crude prostatic fluid (CPF) before being added to the lymphocyte cultures. Exposure of the CPF to antiserum to UG or TG or to an inhibitor of TG, neopentyl chloroethyl nitrosourea (NPCNU), drastically reduced the suppressive effects of CPF (Table 1). In searching for specific CPF factors that may be involved in masking sperm antigenicity, UG and TG were chosen as possible candidates, since both of these proteins are present in CPF. Prostatic fluid in large quantity is difficult to obtain; however, UG is present in abundance in the uterus. There-

Table 1. Suppression of epididymal sperm antigenicity by uteroglobin (UG) and transglutaminase (TG) as measured by [^{3}H]thymidine incorporation into splenocytes. The results are expressed as counts per minute (± standard error of the mean).

Treatment of sperm*	Autologous male		Heterologous male		Female	
	Radioactivity	Percent of control	Radioactivity	Percent of control	Radioactivity	Percent of control
None	9,500 ± 200	100	11,500 ± 350	100	17,000 ± 500	100
Crude prostatic fluid (CPF)	1,320 ± 120	13.9	1,200 ± 115	10	1,800 ± 60	10.5
CPF exposed to antiserum to UG	7,430 ± 260	78.2	8,600 ± 220	74.7	10,800 ± 380	63.5
CPF exposed to NPCNU	6,820 ± 180	71.7	7,400 ± 210	64.3	11,460 ± 370	67.4
CPF exposed to antiserum to TG	8,100 ± 180	85	7,900 ± 210	58.6	11,400 ± 611	67.5
UG	4,200 ± 180	44	6,700 ± 200	58	9,100 ± 150	53.5
TG	8,210 ± 200	86.4	10,800 ± 182	93.9	14,100 ± 210	83.0
UG plus TG	900 ± 65	9.5	760 ± 80	6.6	600 ± 120	3.5
UG, and TG exposed to antiserum to TG	6,300 ± 250	66	8,200 ± 320	71	11,000 ± 210	64.7
UG, and TG inhibited with NPCNU	8,280 ± 110	76	9,800 ± 180	85	12,300 ± 680	72
TG, and UG exposed to antiserum to UG	7,100 ± 150	74	8,500 ± 410	74	18,500 ± 1,150	108
Myoglobin (MG) and TG	8,750 ± 120	92	10,100 ± 220	88	17,200 ± 950	101
Splenocytes and PHA	156,000 ± 1,050	1,642	148,000 ± 2,100	1,287	165,000 ± 1,200	970
Splenocytes only	870 ± 150	9.1	820 ± 201	7.1	695 ± 120	4.0

*The spermatozoa were treated with the indicated components, washed, and incubated with splenocytes in culture as described. The doses of each component, per milliliter, were CPF, 2.1 μg of protein; UG, 2 μg; TG, 3.5 units; MG, 5.0 μg; NPCNU, 1.0 μg; and PHA, 0.1 mg.

fore, this protein was isolated and purified (*6*) from the uteri of rabbits primed with human chorionic gonadotropin (hCG) rather than from the prostatic fluid.

When epididymal spermatozoa were treated with purified UG their antigenicity was reduced, as shown by decreased [^{3}H]thymidine incorporation by the splenocytes (Table 1). When the sperm were treated with UG that had previously been exposed to antiserum to UG, lymphocyte stimulation was not suppressed. Although UG alone had some suppressing effect, dramatic inhibition of sperm antigenicity occurred only when UG and TG were used together. Whereas TG alone had no suppressive effect on sperm antigenicity, the addition of NPCNU, a noncompetitive inhibitor of TG, reduced the ability of UG to suppress lymphocyte stimulation (Table 1). Treatment of TG with its antiserum produced similar results. The antisera to UG and TG, as well as NPCNU, were tested for their effects on the lymphocytes and found to be nonstimulatory. The suppression of splenocyte stimulation by UG-treated spermatozoa was dose-dependent (data not shown). At a concentration of 2 μg/ml (5×10^5 sperm), UG significantly reduced [^{3}H]thymidine incorporation into both autologous and heterologous splenocytes compared to the controls. Total suppression occurred at a concentration of 100 μg/ml (5×10^5 sperm). However, in the presence of TG, only 2 μg of UG per milliliter totally suppressed the antigenicity of epididymal spermatozoa (Table 1). Viability of splenocytes was not affected when UG alone or UG plus TG was added to the cultures.

To ascertain whether or not UG actually bound to the sperm surface, we used an indirect immunofluorescence technique as described previously (*7*). The highest intensity of immunofluorescence occurred on the surface of sperm treated with both UG and TG. Sperm treated with TG that had been exposed to its antiserum or to NPCNU showed no immunofluorescence when compared to sperm treated with UG and TG (Fig. 1c). Treatment of epididymal sperm with CPF resulted in fluorescence; however, exposure of CPF to either NPCNU or antiserum to UG abolished the immunofluorescence (Fig. 1). These results support the suggestion that UG in the prostatic fluid may interact on the sperm surface.

There are at least three mechanisms that may explain the observed lymphocyte suppression of UG plus TG: (i) the sperm antigens may be masked by these proteins; (ii) the sperm antigens may undergo changes in steric conformation; and (iii) the ability of the lymphocytes to recognize antigens may be actively suppressed. The evidence supporting the conclusion that UG in combination with TG masks sperm antigenicity is as follows: UG is present in vas deferens and seminal vesicle (*8*) and TG is present in prostatic fluid (*9*); ejaculated spermatozoa (*4*), but not the epididymal spermatozoa (*10*), are nonimmunogenic and have immunosuppressive properties; the immunogenicity of epididymal sperm is abolished by treatment with CPF or UG and TG; and sexually active females seldom develop antibodies to spermatozoa. The active ingredients in the prostatic fluid that mask the antigens on rabbit spermatozoa may be UG and TG. This is suggested by the data showing that the antigen-masking effects of UG and TG are abolished by treatment with their respective antisera. We recently described a mechanism that might explain this masking process (*11*). In this mechanism, embryonic cell surface antigens may cross-link with UG in the presence of TG and thereby lose their antigenicity. Evidence supporting this hypothesis has been published (*7*). β_2-Microglobulin (β_2M), one of the components of some transplantation antigens and a cell surface protein, is a substrate of TG (*9*). Furthermore, UG in the presence of TG cross-links with certain cell surface proteins (*12*). Thus the formation of cross-linked molecules between antigens containing β_2M (or proteins with amino acid sequences similar to β_2M) and UG present in the prostatic fluid as well as in the uterus of rabbits appears to be possible. It would be of interest to identify such a UG-β_2M complex and to demonstrate experimentally that cell surface antigens in the cross-linked state are immunologically nonrecognizable by their antisera.

Extrapolation of our data, obtained from studies in vitro, to the situation in vivo has inherent difficulties. Whereas TG occurs in all animals, UG has not been detected in animals other than the rabbit. We have investigated only one possible substrate of TG (that is, UG) for its effect on epididymal sperm. There are other proteins in the prostatic fluid that may serve functions very similar to those of UG. The evidence we provide here as well as elsewhere (*7*) supports the notion that UG and TG in the prostate and the pregnant uterus may exert their immunosuppressive effects by masking the cell surface antigens of the male gametes as

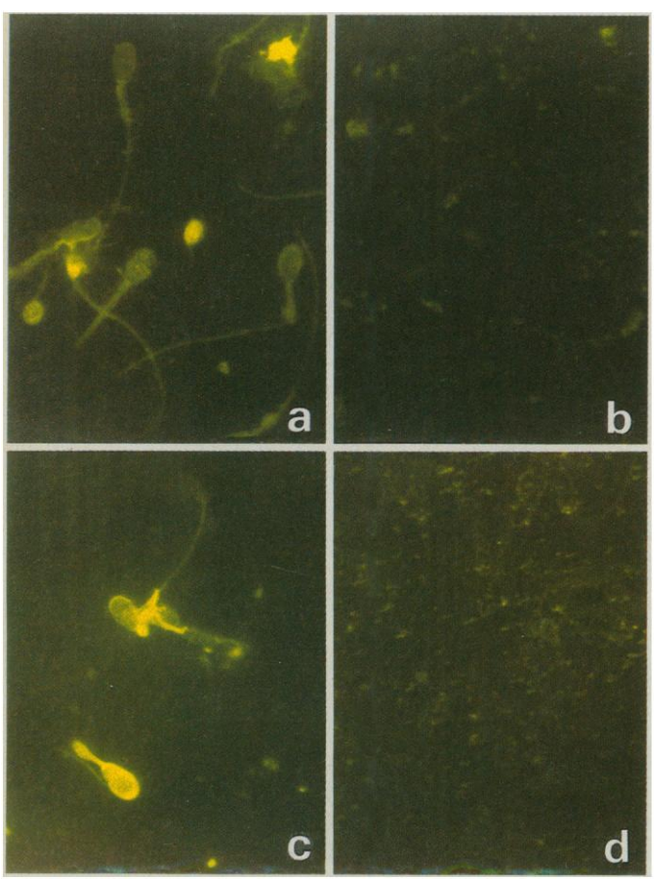

Fig. 1. Indirect immunofluorescence of epididymal spermatozoa (×700). (a) Sperm treated with CPF. (b) Sperm treated with CPF that had been exposed to antiserum to UG. (c) Sperm treated with UG plus TG. (d) Sperm treated with UG plus TG that had been exposed to antiserum to TG.

well as the developing fetus in utero. It is tempting to speculate that a breakdown of the proposed mechanism may lead to infertility and early abortion.

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Action Potentials in Macrophages Derived from Human Monocytes

Abstract. *The electrical activity of macrophages derived from human blood monocytes was recorded in vitro with intracellular microelectrodes and was analyzed with computer-assisted data acquisition and analysis techniques. In cells impaled 6 to 8 days after the cultures were prepared, the resting potentials reached a maximum value of −72 millivolts. The cells were electrically excitable; spikes exhibited a slow upstroke, a fast downstroke, a discrete threshold, a large overshoot, and a brief undershoot. Repetitive firing was induced by a maintained depolarizing current. A positive relation was observed between transmembrane currents and resting potential. Voltage-current relations were nonrectifying for subthreshold current injections. Since these cells had not been treated with any specific activation factors, the electrical activity recorded is evidence for the presence of voltage-dependent inward and outward currents in the membranes of mature macrophages. The electrical signals generated by these cells may be useful for the assay of sensor and effector functions of macrophages, such as chemotaxis, receptor-ligand interactions, and phagocytosis.*

Macrophages exhibit certain of the characteristics of excitable cells, such as transient hyperpolarizations, switching of the resting potential between stable states, and high-resistance regions in N-shaped current-voltage curves (*1–3*). We now report evidence of overshooting, repetitive, spiking behavior in normal human macrophages, and we consider the significance of these events as possible markers of receptor-effector functions.

Human macrophages were prepared from the peripheral blood of normal donors by the Ficoll-Hypaque centrifugation and adherence method (*4*). Mononuclear cells were washed four times with serum-free medium (RPMI-1640, Gibco) to remove platelets. Tissue culture dishes (60 mm^2, Falcon) were each seeded with 4×10^6 cells in 3 ml of complete medium [RPMI-1640 supplemented with gentamicin (50 μg/ml) plus 10 percent autologous serum that was not inactivated with heat]. The cells were incubated at 37°C in 5 percent CO_2 and 95 percent room air for 2 hours. The nonadherent cells were then removed by gentle washing with RPMI-1640, and the adherent cells were fed with complete medium and incubated as above for at least 5 days. The resultant cultures contained more than 95 percent macrophages, as determined by morphology and nonspecific esterase staining (*5*). For studies of electrical activity, cultures were fed with Hepes-buffered (15 m*M*) complete medium, and the culture dish was mounted on a heat-controlled stage of an inverted phase-contrast microscope (Zeiss). Intracellular recordings were carried out with glass microelectrodes filled with 3*M* KCl (40 to 45 megohms) coupled to an electrometer (model KS 900, W-P Instruments) with a Ag-AgCl probe. A similar probe connected the bath to ground. Pulses of constant current were injected into a cell through the recording electrode. Recordings of transmembrane potentials and injected currents were transmitted directly to a microcomputer (New England Digital, Able-40) for storage and analysis.

As the monocyte macrophage became attached to the surface of a plastic culture dish, the cytoplasm spread radially to form an oblate spheroid with a central nucleus. The diameter, or degree of spreading, increased with time. The mean ± standard deviation (S.D.) of the resting potentials recorded from a sample of 28 cells obtained from six donors was −42 ± 14 mV. In this sample, 15 cells exhibited spiking behavior. The voltage-current dependence (*E*/*I*) of the monocyte macrophage membrane was examined by injecting a series of hyperpolarizing and subthreshold depolarizing currents. The slope of the line, representing *E*/*I*, by definition, is the input resistance (R_0) averaged 32 ± 4 megohms. In 85 percent of cells tested ($N = 30$), the resistance to inward and outward test pulses was the same, indicating no rectification. In the rest of the cells, there was slightly less resistance to depolarizing than to hyperpolarizing currents, indicating outward rectification. Estimates of the surface area of the cells averaged 3.93×10^{-5} cm^2 which, when combined with measurements of R_0 (32 ± 4 megohms), gave a calculated value for specific membrane resistance (R_m) of 1258 ohm/cm^2. The membrane time constants were short, seldom ex-