GERM CELL-INDUCED IMMUNE SUPPRESSION IN MICE

Effect of Inoculation of Syngeneic Spermatozoa on Cell-mediated Immune Responses

By U. HURTENBACH* and G. M. SHEARER

From the Immunology Branch of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Autologous sperm are immunogenic and can elicit cell-mediated immunity and antibody responses (1–4). Immune responses against spermatozoa do not occur under normal circumstances, due to the fact that germ cells are efficiently sequestered from the immune system by the blood-testis barrier (5). This barrier, which is established before the immune system becomes competent, creates an immunologically privileged site for sperm and the developing spermatozoa. Therefore, immunological tolerance is not generated to autologous male germ cells. Injuries of the blood-testis barrier (e.g., after vasectomy, infection, or biopsy) can result in the production of autoantibodies and T cell reactivity (1–4).

Because the immune system is not normally exposed to germ cells, it is possible that sperm and other germ cells exert regulatory infuences on immune potential. In the present study, the influence of inoculation of germ cells on cellular immune reactions was investigated. The results indicate that male C57BL/6 mice injected with syngeneic germ cells exhibited: (*a*) reduced natural killer (NK)[1] cell activity; (*b*) reduced mixed lymphocyte reactivity (MLR); (*c*) enhanced auto-proliferation; and (*d*) decreased potential to generate cytotoxic T lymphocyte (CTL) responses to modified self and allogeneic antigens. The reduction in CTL potential against trinitrophenyl-modified syngeneic spleen cells (TNP-self) was mediated by radiosensitive suppressor T cells. These findings are discussed with respect to possible effects of exposure to male germ cells on the immune system.

Materials and Methods

Animals. Young adult mice (8–12-wk) were used for all in vitro sensitization studies. The inbred strains C57Bl6/J (B6), AKR/J, B10.BR (BR), DBA2/J, and (B6 × DBA/2)F_1 (BDF_1) were purchased from The Jackson Laboratory, Bar Harbor, ME.

Cell Preparations. Testicular cell suspensions were prepared as described elsewhere (6). Briefly, testicular (Te) cells were obtained from the seminiferous tubules by protease treatment (7). After removal of the capsule, the testes were incubated at 37°C for 12 min in 0.1% collagenase (Worthington Biochemical Corp., Freehold, NJ), and dissolved in phosphate-buffered saline (PBS). These elements were then broken up by a 15-min incubation at 37°C in 0.025% trypsin (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), dissolved

* Supported by the Deutsche Forschungsgemeninschaft.

[1] *Abbreviations used in this paper:* C, complement; CML, cell-mediated lympholysis; CTL, cytotoxic T lymphocytes; NK, natural killer cell; PBS, phosphate buffered saline; PHA, phytohemagglutinin; SI, stimulation index; Te, testicular cell; TNP, trinitrophenyl.

in Ca^{2+}- and Mg^{2+}-free PBS. This treatment released germ cells of various differentiation stages and Sertoli cells. Sperm were obtained from the epididymis and vasa deferentia. The organs were cut into pieces, minced, and the tissue fragments repeatedly resuspended in PBS plus 2 mM EDTA. The cell suspensions were passed through a sterile nylon screen to remove tissue fragments and cell debris, washed twice in PBS plus EDTA, and injected intravenously into syngeneic recipients. The preparation of spleen cells for in vitro sensitization of CTL has been described previously (8). For in vitro proliferative studies, similar conditions were used, except that the medium was supplemented with 1% heat inactivated normal mouse serum instead of fetal calf serum.

Injection of B6 Mice with Syngeneic Germ Cells. Various numbers of Te cells or sperm suspended in PBS plus EDTA were injected intravenously via the tail vein into syngeneic male recipients. The germ cell donor mice were usually >6 mo.

In Vitro Generation of and Assay for Cellular Immune Reactions. The immune potential of spleen cells from the injected mice was studied 7–52 d after intravenous injection of germ cells. CTL potential was tested by in vitro sensitization against TNP-self and allogeneic spleen cells as described elsewhere (8). The responding cells were cultured with 2,000-rad-irradiated stimulating cells for 5 d. The effector cells generated were tested in a 4-h-^{51}Cr release assay on PHA-stimulated splenic blasts. The percent lysis was calculated as described elsewhere (8). Standard errors of the mean were usually <3% and have been excluded from the graphs for simplicity. The anti-T cell reagent used was a monoclonal anti-Thy-1.2 reagent (New England Nuclear, Boston, MA).

The conditions for the proliferative studies have been reported (6). After in vitro stimulation of the responder cells with 2,000-rad-irradiated allogeneic spleen cells for 4 d, the cultures were pulsed for additional 16–18 h with [^{3}H]thymidine and harvested using a Titertek Multiple Cell Harvester (Flow Laboratories, Rockville, MD). The mean values and the standard deviations were estimated usually from five replicate cultures. Stimulation indices (SI) were calculated as follows: cpm of the mean of the experimental group/cpm of the mean of unstimulated spleen cells.

NK cell activity of spleen cells from the injected mice were tested at various times after inoculation of the spermatozoa. Single spleen cell suspensions were incubated for 4 h on ^{51}Cr-labeled YAC-1 tumor cells. NK cell activity was determined in the same way as CTL activity.

Results

Immune Potential of Mice Injected with Syngeneic Germ Cells. We surveyed NK cell activity, CTL potential to modified self and alloantigens, and, to some extent, proliferative response to alloantigens. In one set of experiments, NK cell activity was tested in B6 mice injected with 1×10^7 syngeneic sperm. Cells from these mice were assayed on YAC-1 tumor cells 8, 22, and 49 d postinoculation. The data of Fig. 1

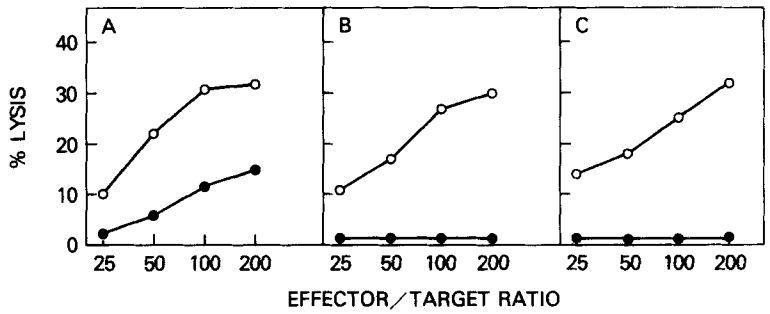

FIG. 1. NK cell activity of B6 spleen cells from mice which were untreated (○) or injected with 1×10^7 syngeneic sperm i.v. (●). NK cell activity was tested on YAC-1 tumor cells 8 (A), 22 (B), or 49 (C) d postinjection.

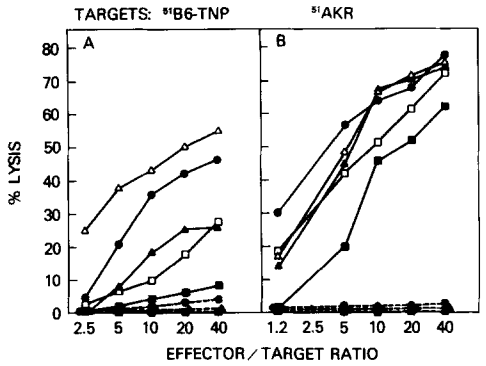

FIG. 2. Cell-mediated cytotoxicity of B6 spleen cells from mice that were untreated (●), or injected 8 d earlier with 1×10^7 B6 sperm (■), 1×10^6 B6 sperm (□), 3×10^7 B6 Te cells (▲), or 1.5×10^7 B6 Te cells (△). Effector cells were generated in vitro against: (A) TNP-self spleen cells and assayed on TNP-self blasts; or (B) AKR spleen cells and assayed on AKR blasts. Broken lines indicated background lysis of the effectors cultured without stimulators.

TABLE I

Reducton of CTL Potential to TNP-Self in BDF_1 Mice by Inoculation of Sperm from Either Parental Haplotype

Anti-BDF_1-TNP-stimulated effector cells	Percent lysis of BDF_1-TNP targets at effector/target ratios shown			
	20:1	10:1	5:1	2.5:1
Normal BDF_1	48	32	31	19
BDF_1 injected with B6 sperm	0	0	0	0
BDF_1 injected with DBA/2 sperm	16	7	6	0

CTL potential was tested 4 d after injection of 15×10^6 B6 or DBA2 sperm i.v.

indicate that 8 d after germ cell injection, NK cell activity was reduced compared with controls, and decreased further with time thereafter.

In other experiments, spleen cells from mice injected with syngeneic testicular cells or sperm were tested for their potential to generate T cell-mediated cytotoxicity in vitro 8 d postinjection. The results of Fig. 2 show the CTL response against TNP-self antigen generated by B6 spleen cells from mice previously injected with syngeneic germ cells. The cytotoxic activity was compared with the response of B6 responder cells from untreated animals. The data indicate that the CTL response was almost completely abrogated in spleen cells from mice injected with 1×10^7 epididymal sperm. In addition, the injection of 1×10^6 syngeneic sperm or 3×10^7 testicular cells decreased, but did not abrogate CTL activity.

The alloreactivity of the same responder cells to AKR stimulators is shown in Fig. 2 B. Compared with normal response, CTL activity of spleen cells from mice previously injected wtth 1×10^7 B6 sperm was reduced approximately fourfold. No appreciable reduction of CTL reactivity was detected under these conditions from mice injected with 1×10^6 sperm, 1.5×10^7, or 3×10^7 Te cells.

Similar effects on CTL reactivity was also observed in BDF_1 recipients, which were inoculated with either 1.5×10^7 B6 or DBA/2 sperm 4 d earlier. As indicated in Table I, the CTL response against BDF_1-TNP stimulators was completely abrogated in spleen cells from mice injected with B6 sperm and was reduced at least 10-fold

after injection of DBA/2 sperm. Identical results were obtained when these effectors were assayed on B6-TNP or DBA/2-TNP targets (data not shown).

To study the kinetics of the depressed CTL potential, B6 mice were injected with 1×10^7 syngeneic sperm i.v. 8, 21, and 52 d later, the spleen cells from these animals were sensitized in vitro to TNP-self or to AKR spleen cells. The results, summarized in Fig. 3, indicate that the CTL activity against TNP-self antigen was strongly reduced by 8 d after injection and was abolished on days 21 and 52. In contrast, alloreactivity was only marginally affected on day 8 (Fig. 3 D), but was decreased as a function of time after inoculation of sperm. By day 52, the allogeneic CTL response was reduced by >30-fold. (Fig. 3 F).

In preliminary experiments, variability was observed in the potential of different sperm preparations to suppress CTL. To determine whether the age of either the donors or the recipients of the sperm is an important factor in eliciting depressed immune potential, two types of age experiments were performed. First, B6 mice of different ages (2, 4, or 9 mo) were injected with 1×10^7 syngeneic sperm from 9-mo-old donors (Table II, experiment 1). The results indicate that first, recipients of all of the three ages tested were effectively suppressed by sperm from 9-mo-old donors, although suppression might be slightly more pronounced in older recipients. Second, sperm from 9-mo- but not from 2.5-mo-old donors were effective in reducing immune potential, irrespective of the age of recipients (experiment 2).

Microscopic examination of the sperm inoculum indicated that the preparation also contained other somatic cells (at a concentration of approximately one-half the number of sperm). To test whether certain other syngeneic somatic cells would reduce

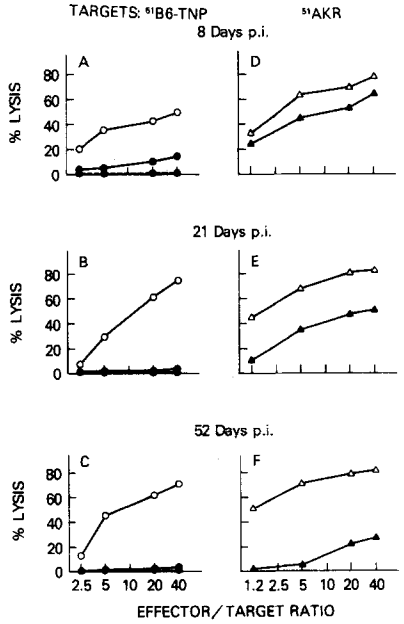

FIG. 3. Kinetics of the depressed CML reactivity in spleen cells from B6 mice injected with 1×10^7 syngeneic sperm. Effector cells were generated by in vitro sensitization against TNP-self (A–C) or AKR (D–F) spleen cells 8, 21, or 52 d postinjection (p.i.) of the sperm and assayed on the specific targets. (○, △): effector cells derived from untreated mice; (●, ▲): effector cells derived from sperm-injected mice. Broken lines indicated background lysis of the effectors cultured without stimulators.

TABLE II

Effect of Age of Sperm Recipients and Donors on Depressed CTL Potential to TNP-Self

Anti-B6-TNP effectors from mice:	Percent lysis of TNP-modified B6 targets at effector/target ratio		
	40:1	20:1	5:1
Experiment 1			
2-mo-old:			
Uninjected	55	32	2
Injected	20	5	1
4-mo-old:			
Uninjected	45	26	11
Injected	11	8	0
9-mo-old			
Uninjected	58	51	20
Injected	5	3	1
Experiment 2			
Uninjected	66	60	42
Injected with sperm from:			
2.5-mo-old donors	70	67	47
9-mo-old donors	30	22	10

CTL potential was tested 14 d after injection of 1×10^7 syngeneic sperm.

TABLE III

Effect of Sperm and Lymphoid Cells from Aged Donors on CTL Potential of Syngeneic Recipients

Anti B6-TNP-stimulated effectors	Percent lysis of B6-TNP targets at effector/target ratios shown			Anti-BR-stimulated effectors	Percent lysis of BR targets at effector/target ratios shown		
	40:1	20:1	5:1		20:1	5:1	1.2:1
Normal B6	35	18	2	Normal B6	73	52	22
B6 injected with sperm cells	7	2	1	B6 injected with sperm cells	52	35	6
B6 injected with spleen cells	27	19	5	B6 injected with spleen cells	65	51	12

CTL potential was tested 5 d after injection of 2×10^7 syngeneic sperm or 2×10^7 syngeneic spleen cells from 12-mo-old B6 donors.

CTL potential, an experiment was performed in which 2×10^7 B6 sperm or 2×10^7 B6 spleen cells were inoculated into syngeneic recipients. 5 d later the CTL potential of the mice was tested. In contrast to the immunosuppressive effect of sperm, spleen cells from the same donors did not affect CTL responses (Table III).

To verify the immunosuppressive effect of syngeneic sperm, the proliferative responses of B6 recipient mice were tested. MLR in vitro was determined by [^{3}H]-thymidine uptake after 5 d of cultivation. The results are shown in Table IV. Spleen cells from mice injected with 1×10^7 syngeneic sperm 7 d earlier exhibited decreased alloreactivity against AKR simulator cells when assayed for proliferation (expressed as SI). However, in terms of absolute counts per minute, no appreciable reduction of alloreactivity was detected 7 d after injection of germ cells. In contrast, when spleen cells from mice inoculated with 1×10^7 sperm were tested after 49 d, considerable

TABLE IV

[^{3}H]Thymidine Incorporation of Spleen Cells from Mice Injected with Syngeneic Germ Cells

Responder cells from B6 mice	Stimulator cells		SI
	B6 Spleen cells	AKR spleen cells	
	cpm ± *SE*	*cpm* ± *SE*	
Experiment 1*			
Untreated	2,685 ± 134	37,789 ± 2,079	14.1
Injected with 1 × 10^7 sperm	4,262 ± 218	28,992 ± 1,856	6.8
Experiment 2‡			
Untreated	3,208 ± 442	37,717 ± 3,658	11.7
Injected with 1 × 10^7 sperm	8,107 ± 264	19,437 ± 2,018	2.4

* Spleen cells tested 7 d after injection.
‡ Spleen cells tested 49 d after injection.

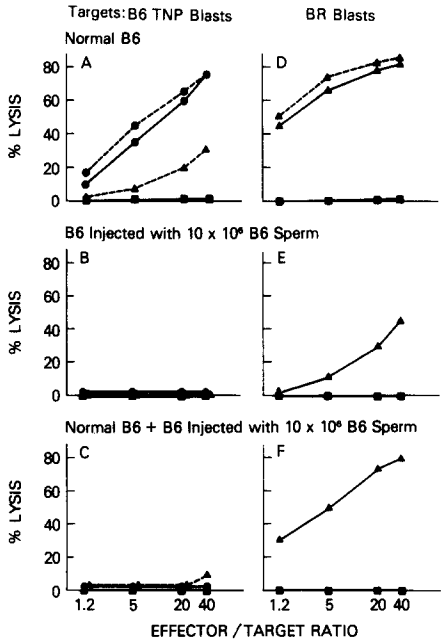

FIG. 4. Cell-mediated cytotoxicity of B6 spleen cells to TNP-self (A–C) or BR (D–F) stimulator cells. Effector cells were generated by in vitro sensitization of spleen cells from untreated mice (A, D; 4 × 10^6 cells/well), mice injected with 10 × 10^6 B6 sperm i.v. 22 d earlier (B, E; 4 × 10^6 cells/well), and or by co-culturing 4 × 10^6 spleen cells from untreated with 4 × 10^6 spleen cells from injected mice (C, F). 8 × 10^6 (●- - -●) or 4 × 10^6 (●——●) cultured spleen cells were stimulated against TNP-self and assayed on TNP-self targets; 8 × 10^6 (▲- - -▲) or 4 × 10^6 (▲——▲) spleen cells were stimulated against BR spleen cells and tested on BR targets. (■) indicates background lysis of the effectors cultured without stimulators.

decrease of the alloreactivity was observed. Significant levels of unstimulated proliferation (autoproliferation) was detected in spleen cells from all groups of animals.

Demonstration of Suppressor Activity in Spleen Cells from Mice Injected with Syngeneic Sperm. Co-culture experiments were performed to test whether the inoculation of syngeneic germ cells resulted in the generation of suppressor cells. 4 × 10^6 normal B6 reponder

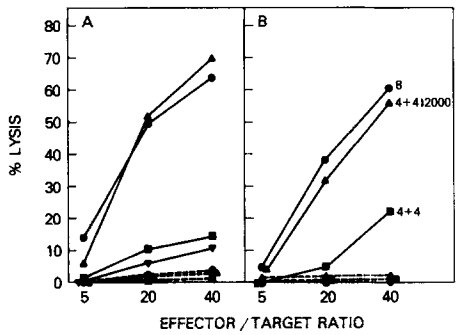

FIG. 5. Cell-mediated cytotoxic responses of B6 spleen cells against TNP-self. Effector cell activity of 8×10^6 normal spleen cells (●) (A, B). Effector cell activity of A: 4×10^6 normal spleen cells plus 4×10^6 spleen cells from mice injected 14 d earlier with 10×10^6 B6 sperm (■); 4×10^6 normal spleen cells plus 4×10^6 spleen cells from sperm-injected mice treated with C′ (▼); 4×10^6 normal spleen cells plus 4×10^6 spleen cells from sperm-injected mice treated with anti-Thy-1 and C′ (▲). Effector cell activity of B: 4×10^6 normal spleen cells plus 4×10^6 spleen cells from mice injected 13 d earlier with 10×10^6 B6 sperm (■); 4×10^6 normal spleen cells plus 2,000-rad-irradiated 4×10^6 spleen cells from sperm-injected mice (▲). Broken lines indicate background lysis of effectors cultured without stimulators.

cells were co-cultured with 4×10^6 spleen cells from mice injected with 1×10^7 B6 sperm 3 wk earlier. The responder populations were sensitized in vitro against TNP-self or allogeneic BR stimulator cells (Fig. 4). The results of Fig. 4 B demonstrate that responder cells from the injected mice were depressed in the CTL activity against TNP-self antigens. Furthermore, the anti-TNP-self response of normal B6 responder cells was suppressed by co-culture of spleen cells from injected mice (Fig. 4 C). Similarly, the cross-reactive lysis of BR alloantigen by effector cells generated against B6-TNP stimulator cells was decreased (Fig. 4 C). In contrast, spleen cells from the injected animals failed to appreciably suppress the allogeneic CTL activity of normal B6 spleen cells against BR stimulators (Fig. 4 F), although responding cells from the injected mice generated poorer allogeneic cell-mediated lysis (CML) (Fig. 4 E).

Partial Characterization of Suppressor Cell Activity. Spleen cells from mice injected with 1×10^7 syngeneic sperm 8 d previously were exposed to 2,000 rad irradiation or to anti-Thy-1.2 plus complement (C′) treatment. Subsequently, their suppressor activity was tested by co-culturing with normal B6 responder cells. Fig. 5 A demonstrates that suppression of the TNP-self response was eliminated after exposing the spleen cells from the injected animals to 2,000 rad. Moreover, anti-Thy-1.2 plus C′ treatment also abolished suppressor activity (Fig. 5 B).

Discussion

The present study investigates the immune reactivity of mice previously injected with syngeneic male germ cells. The experiments indicate that spleen cells from such mice exhibited reduced immune function as determined by: (*a*) NK cell activity; (*b*) MLR; and (*c*) the potential to generate T cell-mediated cytotoxicity. This effect was detected as early as 4 d after injection of the germ cells, and persisted for >7 wk. The decrease in the CTL response to TNP-self was attributed to suppression, because spleen cells from the injected animals effectively inhibited the immune response of normal syngeneic responder cells. This suppressor activity was sensitive to anti-Thy-1 plus C′ treatment and to irradiation with 2,000 rad, and thus was mediated by T

cells. Depression of CTL potential appeared to be antigen nonspecific, as both TNP-self and allogeneic CTL responses were affected. Early depression to TNP-self response was more marked than that to the alloantigens. At longer intervals after germ cell injection, stronger reduction of the allogeneic response was observed, and was associated with more pronounced depression of TNP-self response. These differences in timing of induction of depressed CTL function to TNP-self and alloantigen and the apparent differences in our ability to demonstrate suppression by in vitro cell mixing experiments could be due to: (*a*) the fact that hapten-self responses are generally weaker than allogeneic responses; (*b*) the possibility that suppression is demonstrable ony for foreign antigen plus self, but not for allogeneic CTL responses (despite the fact that both types of responses are depressed *in situ*), or our not having defined the conditions for demonstrating in vitro suppression for allogeneic responses. Irrespective of the apparent differences in demonstrating suppression, the more important point is that both CTL responses were abrogated in mice injected with syngeneic sperm. We observed some variability in the induction of suppression with spermatozoa. One factor found to be critical for inducing depressed cellular immune function was the age of the sperm donors. Depressed CTL function was readily induced in both young and old mice with syngeneic germ cells from old mice. However, germ cells from young animals were ineffective in reducing immune responses. These observations indicate that suppressor-inducing properties of male germ cells are age dependent, which raises the possibility that the differences are hormonally mediated and could be related to differential antigen expression (auto-, embryonic, or viral in nature) on germ cells. In addition, sperm were much more effective than Te cells, suggesting that more mature stages of germ cells induce suppression.

A number of mechanisms could be envisioned for the generation of suppressor cells by syngeneic germ cells. For example, the CTL suppression observed here might be due to seminal plasma introduced into the recipient mice. Suppression of proliferative T cell reactivity in vitro by sperm and seminal plasma has been reported (9–11), and it has been suggested that such suppression mediated by sperm is in fact due to seminal plasma antigens coating the spermatozoa (11, 12). This explanation seems to be unlikely in the present studies, because the sperm preparations were washed thoroughly before injection. Moreover, Te cells, which are not exposed to seminal fluid, also induce suppression. Alternatively, viral antigens expressed on germ cells (13) might induce immune suppression (14, 15). If this were true, one would expect spermatozoa to express more viral antigens than testicular cells, as the former were more suppressive. It has been reported that more glycoprotein 70 was detected on epididymal cells than on the seminiferous tubules (13). Suppression induced by viral antigens on sperm cannot, therefore, be completely excluded. It is also possible that synergistic effects of virus infection and sperm could lead to suppression. The mouse colony in which the recipients and donors were housed was screened quarterly for a number of murine pathogenic viruses (including lymphocytic choriomeningitis, cytomegalovirus, ectromelia, murine hepatitis, and Sendai). The colony consistently exhibited antibody titers against Sendai, and occasionally weak titers against hepatitis. A third possible suppressive mechanism could be attributed to embryonic antigens expressed by germ cells (16). Many reports have demonstrated the suppressor-inducing activity of embryonic cells or embryonic antigens (17–19). Thus, it it conceivable that embryonic antigens on germ cells regulate the induction of suppressor cells. It is also

possible that germ cells activate suppressor T cells due to their haploid nature, or to the fact that they are normally sequestered from the lymphoid system. Although it is difficult to know what cells would serve as appropriate negative controls in this system, the injection of syngeneic hemopoietic or lymphoid elements does not result in suppression. It should also be noted in this context that although some syngeneic lympocytes ($2–5 \times 10^6$) are injected with the 10^7 sperm inoculum, it is unlikely that they could have contributed to suppression, because the injection of an equivalent number of syngeneic lymphocytes had no effect on cellular immune function.

Even after inoculation of BDF_1 recipients with sperm from either parental haplotype, the possibility that suppression is due to contaminating parental lymphocytes in the inoculum can be excluded because: (*a*) BDF_1 mice are naturally resistant to suppression induced by even 2×10^7 parental spleen cells (20); and (*b*) the contaminating somatic cells in the sperm inocula all stained with trypan blue and were probably killed by the relatively high centrifugation speed used in washing the sperm suspension.

The results of the present study suggest that exposure of the germ cells or their antigens to the immune system (e.g., after vasectomy) could lead to serious immunpathological side effects. In fact, antisperm antibodies develop after vasectomy in a large percentage of men and experimental animals (21), and are associated with histopathological changes within the testes and epididymis (22), the ductus efferentes (23), the basement membrane of the renal glomeruli (24), and in the arteries (25). Moreover, impaired cellular immune responses have been reported following vasectomy of rhesus monkeys (26) and guinea pigs (27), but were not observed in mice (22). So far, no serious clinical consequences have been reported after vasectomy of humans (28). However, careful studies of the possible long-term effects of vasectomy on specific immune responses of humans remain to be performed.

Finally, it is possible that the sperm-induced T cell-mediated suppression of CTL and NK function demonstrated in the present study is related to recent reports of T cell and NK dysfunction in homosexual men who have had an abnormally high incidence of *Pneumocystis carinii* pneumonia, *Candida albicans*, multiple viral infections, and Kaposi's sarcoma (29–31). These studies have postulated that the immune suppression observed could be a result of infection with cytomegalovirus and/or other agents (29–31). It is noteworthy in this context that mice injected with allogeneic spermatozoa are also immune suppressed (U. Hurtenbach, unpublished observations). We raise the possibility that sperm may reach the blood stream of these individuals via intestinal mucosa lesions and that, particularly after repeated exposure, these patients may experience severe immunosuppression. This could be induced by sperm, which would make them more susceptible to virus infections, or synergistically, by repeated exposure with low doses of sperm plus viruses and/or drugs.

Summary

Spleen cells from mice injected intravenously with syngeneic male germ cells exhibited reduced immune functions as determined by natural killer cell activity, mixed lymphocyte reactivity and cytotoxic lymphocyte (CTL) function. The decrease in CTL responses to trinitrophenyl-modified self (TNP-self) was detected as early as 4 d after sperm injection and was observed to H-2 alloantigens 3 wk after injection. Radiosensitive suppressor T cells were found to suppress the CTL response to TNP-

self. Suppression lasted for a period of at least 7 wk after a single inoculation of the germ cells. Some variability in immune suppression capability was observed using different preparations of germ cells which are not yet completely understood. Sperm were more effective in inducing suppression than testicular cells derived from the seminiferous tubules. Furthermore, sperm from older animals were more effective than those from younger mice. These findings are discussed with respect to possible regulatory influences of germ cells on the immune system when the blood-testes barrier is broken.

The authors express their appreciation to Dr. Richard Hodes, Dr. Stephen Shaw, and Dr. William Terry for reviewing the manuscript and for helpful suggestions, and to Ms. Judy Kress for preparing the manuscript.

Received for publication 11 January 1982.

References

1. Cochrane, C. G., and D. Koffler. 1973. Immune complex disease in experimental animals and man. *Adv. Immunol.* **16:**186.
2. Johnson, M. H. 1973. Physiological mechanisms for the immunological isolation of spermatozoa. *Adv. Reprod. Physiol.* **6:**279.
3. Tung, K. S. K., and N. A. Alexander. 1976. Autoimmune reactions in the testis. *In* The Testis. A. D. Johnson and W. R. Gomes, editors. Academic Press Inc., New York. **IV:**33–91.
4. Law, H. Y., W. F. Bodmer, J. D. Mathews, and D. C. G. Skegg. 1979. The immune response to vasectomy and its relation to the HLA system. *Tissue Antigens.* **14:**115.
5. Dym, M., and D. W. Fawcett. 1970. The blood testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod.* **3:**308.
6. Hurtenbach, U., F. Morgenstern, and D. Bennett. 1980. Induction of tolerance in vitro by autologous murine testicular cells. *J. Exp. Med.* **151:**827.
7. Romrell, L. J., A. K. Bellve, and D. W. Fawcett. 1976. Separation of mouse spermatogenic cells by sedimentation velocity. A morphological characterization. *Dev. Biol.* **49:**119.
8. Schmitt-Verhulst, A. M., C. B. Pettinelli, P. A. Henkart, J. K. Lunney, and G. M. Shearer. 1978. H-2 restricted cytotoxic effectors generated in vitro by the addition of trinitrophenyl-conjugated soluble proteins. *J. Exp. Med.* **147:**352.
9. Lord, E. M., G. F. Sensabaugh, and D. P. Stites. 1977. Immunosuppressive activity of human seminal plasma. I. Inhibition of in vitro lymphocyte activation. *J. Immunol.* **118:**1704.
10. Marcus, Z. H., J. H. Freisheim, J. L. Houk, J. H. Herman, and E. V. Hess. 1978. In vitro studies in reproductive immunology. 1. Suppression of cell mediated immune response by human spermatozoa and fractions isolated from human seminal plasma. *Clin. Immunol. Immunopathol.* **9:**318.
11. Stites, D. P., and R. P. Erickson. 1975. Suppressive effect of seminal plasma on lymphocyte activation. *Nature (Lond.).* **253:**727.
12. Prakash, C., A. Coutinho, and G. Moller. 1976. Inhibition of in vitro immune responses by fraction from seminal plasma. *Scand. J. Immunol.* **5:**77.
13. Lerner, R. A., C. B. Wilson, B. C. Del Villano, P. J. McConah, and F. J. Dixon. 1976. Endogenous oncorna-viral gene expression in adult and fetal mice: quantitative, histological and physiological studies of the major viral glycoprotein gp 70. *J. Exp. Med.* **143:**151.
14. Caulfield, M. J., and J. Cerny. 1980. Cell interactions in leukemia associated immunosuppression: suppression of thymus-independent antibody responses by leukemia spleen cells (Moloney) in vitro is mediated by normal T cells *J. Immunol.* **124:**255.

15. Reinherz, E. L., C. O'Brien, P. Rosenthal, and S. F. Schlossman. 1980. The cellular basis for viral induced immunodeficiency: analysis by monoclonal antibodies. *J. Immunol.* **125:**1269.
16. Gachelin, G. M. Fellous, J. L. Guenet, and F. Jacob. 1976. Developmental expression of an early embryonic antigen common to mouse spermatozoa on cleavage embryos and to human spermatozoa: its expression during spermatogenesis. *Dev. Biol.* **50:**310.
17. Globerson, A., and T. Umiel. 1978. Ontogeny of suppressor cells. II. Suppression of GVH and mixed leukocyte culture response by embryonic cells. *Transplantation (Baltimore).* **26**(Suppl 6):438
18. Murgita, R. A., J. A. Andersson, M. S. Sherman, H. Bennich, and H. Wigzell. 1978. Effects of human α-fetoprotein on human B and T lymphocyte proliferation in vitro. *Clin. Exp. Immunol.* **33:**347.
19. Bluestone, J. A., C. Lopez, and U. Hurtenbach. 1981. Mixed lymphocyte reactivity in spleen cells from F-9 tumor-bearing mice. I. Suppressor cell induction and characterization. *Cell. Immunol.* **62:**334.
20. Shearer, G. M., and R. P. Pollison. 1980. Mutual recognition of parental and F_1 lymphocytes. Selective abrogation of cytotoxic potential of F_1 lymphocytes by parental lymphocytes. *J. Exp. Med.* **151:**20.
21. Alexander, N. J., and D. J. Anderson. 1979. Vasectomy: consequences of autoimmunity to sperm antigens. *Fertil. Steril.* **32:**253.
22. Anderson, D. J., and N. J. Alexander. 1981. Antisperm antibody titres, immune complex deposition and immunocompetence in long-term vasectomized mice. *Clin. Exp. Immunol.* **43:**99.
23. Alexander, N. J., and S. K. Tung. 1979. Vasectomy in the rabbit: immunological and morphological effects. *In* Vasectomy. Immunological and Pathological Effects in Animals and Man. I. H. Lepow and R. Crozier, editors. Academic Press, Inc., New York. 355–377.
24. Bigazzi, P. E., L. L. Kosuda, K. C. Hsu, and G. A. Andres. 1976. Immune complex orchitis in vasectomized rabbits. *J. Exp. Med.* **143:**382.
25. Alexander, N. J., and T. B. Clarkson. 1978. Vasectomy increases the severity of diet induced atherosclerosis in mucosa fascicular. *Science (Wash. D. C.).* **201:**538.
26. Wilson, B. J., G. Porter, and A. Goldstein. 1979. Reduced T cell reactivity in vasectomized rhesus monkeys: association with histocompatibility type. *Fertil. Steril.* **31:**434.
27. Muir, V. Y., and J. L. Turk. 1979. Immunological nonresponsiveness during induction of experimental autoimmune orchitis in guinea pigs: studies in vivo and in vitro. *Immunology.* **36:**95.
28. Survey of vasectomy (editorial). 1979. *Lancet.* **II:**1057.
29. Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon. 1981. *Pneumocystis carinii* pneumonia and mucosal cardidiasis in previously healthy homosexual men. *New Engl. J. Med.* **305:**1425.
30. Masur, H., M. A. Michelis, J. B. Greene, I. Ouorato, R. A. V. Stouwe, R. S. Holzman, G. Wormser, L. Brettman, M. Lange, H. W. Murray, and S. Cunningham-Rundles. 1981. An outbreak of community-acquired *Pneumocystis carinii* pneumonia. Initial manifestitation of cellular immune dysfunction. *New Engl. J. Med.* **305:**1431.
31. Siegal, F. P., C. Lopez, G. S. Hammer, A. E. Brown, S. J. Kornfeld, J. Gold, J. Hassett, S. Z. Hirschman, C. Cunningham-Rundles, B. R. Adelsberg, D. M. Parham, M. Siegal, S. Cunningham-Rundles, and D. Armstrong. 1981. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *New Engl. J. Med.* **305:**1439.