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Sperm Autoantibodies in Vasectomized Rats of Different Inbred Strains

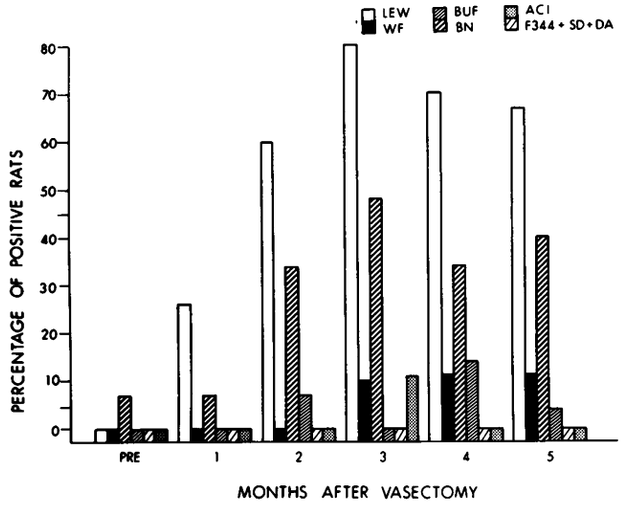
Abstract. *An immune response to antigens of spermatozoa occurs after vasectomy in rats of some inbred strains, but not in others. Antibodies to rat spermatozoa were detected by indirect immunofluorescence in some of the serums of vasectomized rats of the following strains: 80 percent of Lewis, 47 percent of Brown Norway, 13 percent of Buffalo, 12 percent of Wistar-Furth, and 11 percent of ACI rats. No such antibodies were detected in the serums of vasectomized Fischer, Dark Agouti, and Sprague-Dawley rats.*

Results of studies on the effects of vasectomy in rats are not uniform, some describing macroscopic or microscopic changes (or both) in the testes of vasectomized animals (*1*), others reporting no alterations (*2*). This disparity is not surprising, since these investigations were performed with different strains of outbred rats, and the results are therefore difficult to compare. Recent investigations have demonstrated that vasectomy may be followed by an autoimmune response to antigens of spermatozoa (*3*) and, in rabbits, by an immune complex-mediated orchitis (*4*). It has also been shown that rats, like mice and guinea pigs, possess histocompatibility-linked immune response genes (*5*) and that the appearance of some experimentally induced autoimmune diseases is under genetic control (*6–8*). For these reasons, we have investigated the production of antibodies to antigens of spermatozoa in bilaterally vasectomized rats of different strains, namely, inbred Lewis (LEW), Brown Norway (BN), Buffalo (BUF), ACI, Wistar-Furth (WF), Fischer (F344), Dark Agouti (DA), and outbred Sprague-Dawley rats (*9*).

A maximum of 80 percent of vasectomized LEW rats had circulating antibodies to rat spermatozoa, detectable by indirect immunofluorescence (IF) on sperm smears (*10*) (Fig. 1). Similar antibodies were also found in the serums of 47 percent of BN, 13 percent of BUF, 12 percent of WF, and 11 percent of ACI rats that had been vasectomized. In contrast, vasectomized F344, DA, and Sprague-Dawley rats did not have antibodies to sperm in their serums. The incidence of antibodies to spermatozoa in serums of vasectomized rats increased with time, reaching its maximum at 3 months after vasectomy in LEW, BN, and ACI and at 4 months in BUF and WF (Fig. 1).

Positive reactions obtained with LEW, BN, ACI, BUF, and WF serums were localized in the acrosomal region of rat spermatozoa (Fig. 2) and were obtained with sperm smears from LEW, BN, ACI, BUF, and WF as well as F344, DA, and Sprague-Dawley rats. This result indicates that acrosomal antigens involved in this immune response are present even in the spermatozoa of those rats like F344, DA, and Sprague-Dawley that did not produce antibodies to spermatozoa. The antibodies to sperm in the serums of positive animals were capable of reacting with spermatozoa from animals of the same inbred strain and also with spermatozoa from the positive animals themselves; thus they can be defined as autoantibodies.

Titers of antibodies to acrosomal antigens of spermatozoa ranged from 10 (or less than 10) to 320. The mean titers were 137 ± 39 in positive serums of LEW rats and 48 ± 30 in positive serums of BN rats. The few positive animals in the other strains had titers of 10 or less than 10. Serums from sham-vasectomized and untreated rats as well as serums from prevasectomy bleedings obtained from LEW, ACI, BUF, and WF rats did not contain antibodies to rat spermatozoa. The prevasectomy serum from one BN rat gave a positive reaction, with a titer



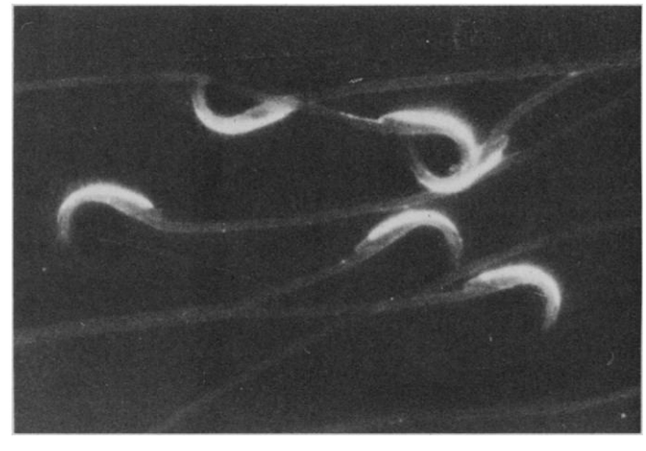


Fig. 1 (left). Incidence and time sequence of sperm antibody response in bilaterally vasectomized rats of different strains. Abbreviations: *LEW*, Lewis; *WF*, Wistar-Furth; *BUF*, Buffalo; *BN*, Brown Norway; *F344*, Fisher; *SD*, Sprague-Dawley; *DA*, Dark Agouti. Fig. 2 (right). Demonstration of antibodies to the acrosome of rat spermatozoa by indirect immunofluorescence (photographed at 100×).

of 80. The serum of this animal was found to be positive for sperm antibodies until 7 months after vasectomy, with titers lower than in the prevasectomy serum. So-called "natural" antibodies to spermatozoa have been detected in the serums of mice, guinea pigs, rabbits, and human beings; and their production has been explained on the basis of cross-reactions with exogenous antigens, possibly present in microorganisms (*11*).

Our findings show that different strains of rats differ in the vasectomy-elicited response to sperm antigens. A large percentage of vasectomized LEW rats and a considerable percentage of BN rats produced antibodies to spermatozoa after vasectomy as compared to smaller numbers of rats from other inbred strains. Rats from three strains, two inbred and one outbred, did not show any immune response to spermatozoa detectable by indirect IF. Antibody titers were elevated in both LEW and BN rats, but consistently low in all other animals. On this basis we may consider LEW and BN rats as high responders to sperm antigens, whereas the other strains may be considered poor responders. These differences are not easily explained on the basis of a simple correlation between major histocompatibility loci and immune response genes controlling autoimmune responses to sperm antigens. Rats that may be considered good responders such as the LEW and the BN belong to different genotypes, Ag-B1 and Ag-B3. In addition, two strains of rats belonging to the same Ag-B1 genotype differed in their response; that is, LEW rats produced antibodies and F344 did not. Previous studies on the genetic control of autoimmune responses in rats have given contrasting results.

Gasser *et al.* (*6*) observed that the genetic locus controlling susceptibility to experimental allergic encephalomyelitis is closely linked to the Ag-B histocompatibility locus, but is not identical to it. Rose (*7*) noted that immune responsiveness in terms of antibody formation to thyroglobulin does not appear to be linked to Ag-B antigens, whereas Ag-B4 and Ag-B2 may be linked to the allele favoring susceptibility to experimentally induced thyroid damage. Finally, an increased susceptibility to Heymann nephritis has been found to be linked to the Ag-B1 locus (*8*). All these investigations have relied on the injection of heterologous antigens or autoantigens in adjuvants. In contrast, our observations of differences in the immune response of different rat strains to self-antigens of spermatozoa are based on a simple surgical procedure (vasectomy) and do not involve the administration of adjuvants. This might prove a definite advantage for further studies of the role of histocompatibility-linked immune response genes in autoimmune reactions.

In any case, the contrasting reports on the effects of vasectomy in rats may be explained by the observation that rats from different inbred strains have a different incidence and intensity of immune responses to sperm antigens. In this regard it should be noted that, although Sprague-Dawley rats are frequently used in vasectomy studies, these animals were completely negative for sperm antibodies.

The immunologic response to sperm antigens in vasectomized rats might have pathological consequences. Damage to the testes in animals with antibodies to spermatozoa might be mediated by immune complex formation at the level of the basement membrane of seminiferous tubules, as observed in vasectomized rabbits (*4*). Alternatively, damage could be mediated by other immunologic mechanisms, such as cytotoxic antibodies, antibody-dependent lymphocyte-mediated cytotoxicity or cytotoxic lymphokines. In this context, it is interesting that cytotoxic antibodies to spermatozoa have been detected in the serums of vasectomized LEW rats (*12*) and that experimental autoimmune orchitis has been induced with relative ease in LEW rats (*13*).

In conclusion, our findings suggest that vasectomy studies performed on the appropriate inbred strains of rats may provide further understanding of the effects of this surgical procedure and at the same time help in clarifying the relation between histocompatibility antigens and autoimmunity.

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9. A total of 11 LEW, 10 F344, 10 WF, 10 ACI, 15 BN, 10 DA, 27 BUF, and 10 Sprague-Dawley rats were vasectomized. Sham vasectomies were performed on ten rats from each of the different strains. Rats were purchased from Microbiological Associates, Walkersville, Md., with the exception of BUF rats, which were obtained from Simonsen Laboratories, Gilroy, Calif., Sprague-Dawley rats, which were obtained from ARS Sprague-Dawley, Madison, Wis., and DA rats, which were raised in our animal unit. Bilateral vasectomies were performed by sterile techniques; trauma to the testes and their vessels was avoided. Rats of approximately 3 months of age and weighing 300 g were trial-bled and then injected with chlorpromazine hydrochloride (25 mg/kg, intramuscularly) and ketamine hydrochloride (40 mg/kg, intramuscularly). Each vas deferens was isolated through a vertical incision (about 1.5 cm long) through the skin of the scrotum on either side and a small incision through the underlying layers. The deferens was ligated in two sites with nonresorbable silk sutures, and the part between the sutures, approximately 1 cm long, was cut out. None of the animals thus vasectomized became cryptorchid. Sham vasectomies were performed in a similar fashion, with the exception that the vas deferens was not ligated and cut out, but left intact. All rats were bled regularly every 30 days from the retroorbital plexus and serums were stored at −70°C.
10. Indirect IF was performed as described (*4*) on smears of spermatozoa obtained from the epididymis of LEW, WF, and other rats after unilateral or bilateral orchiectomy. Smears were prepared with a cytocentrifuge (Cytospin, Shandon-Elliott, Shandon Instruments Inc., Sewickley, Pa.) at 1500 rev/min for 10 minutes, dried in air, fixed in absolute methanol for 10 minutes at room temperature, incubated for 30 minutes at room temperature with twofold serial dilutions of rat serum, starting from a dilution of 1:10 to 1:640. Smears were then washed in phosphate-buffered saline, incubated for 30 minutes with fluorescein-conjugated rabbit antiserum to rat immunoglobulins (Lot 9017, Cappel Laboratories, Downington, Pa.), washed in phosphate-buffered saline, mounted in buffered glycerol, and observed under a Leitz Dialux fluorescence microscope. Reactions were scored on an arbitrary and subjective scale as −, ±, +, 2+, and 3+. In titrations the endpoint was the highest dilution of the serum that gave an unequivocal positive (+) reaction. Controls included sperm smears incubated with prevasectomy serums from the rats tested, serums obtained from sham-vasectomized and untreated rats, and the fluorescein-conjugated antiserum alone.
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