Reversible Immunocontraception in Male Monkeys Immunized with Eppin

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Various forms of birth control have been developed for women; however, there are currently few options for men. The development of male contraceptives that are effective, safe, and reversible is desired for family planning throughout the world. We now report contraception of male nonhuman primates (*Macaca radiata*) immunized with Eppin, a testis/epididymis-specific protein. Seven out of nine males (78%) developed high titers to Eppin, and all of these high-titer monkeys were infertile. Five out of seven (71%) high–anti-Eppin titer males recovered fertility when immunization was stopped. This study demonstrates that effective and reversible male immunocontraception is an attainable goal. This method of immunocontraception may be extended to humans.

Although several different choices and approaches are available for contraception in women, the choices for men are currently limited to condoms and vasectomy (*1*, *2*). Male hormonal contraceptives (*3*, *4*) developed over the past several years have now advanced to clinical trials, and the outcome of these studies may determine whether the suppression of sperm production through androgen regulation can become a realistic product. Immunocontraception, an alternative nonhormonal method, has been studied for many years (*1*, *5*), with the major emphasis on immunization of females to prevent pregnancy (*6*) or fertilization (*7*). In the present study, we report the successful contraception of male nonhuman primates (*M. radiata*) immunized with Eppin, a testis/epididymis-specific protein (*8*, *9*). This represents a non–hormonally disruptive male immunocontraceptive for primates.

Before using the monkey as a model for the test of a male immunocontraceptive, we determined the presence of both Eppin and immunoglobulin (IgG) in the male reproductive tract, the immunogenicity of Eppin, and the effects of immunization on sperm motility at the University of California, Davis (UC-Davis). Results from these studies are shown in figs. S1 to S3 and the supporting online material text (*10*). The studies on normal *Macaca* monkeys at UC-Davis allowed us to determine that IgG is present in the normal epididymal tract. Moreover, immunization of two monkeys at UC-Davis indicated that the immune response to recombinant Eppin could influence the titer of antibodies to Eppin in their semen. These results are similar to a previous study in female macaques demonstrating that different serum titers resulted in correspondingly different antibody titers in oviductal fluid (*11*). Consequently, in the fertility study described below, two male monkeys in the initial immune group were dropped because they could not sustain a high serum titer and were unlikely to have a high semen titer. The lack of a strong immune response to an immunogen in a particular individual animal is a reflection of the major histocompatibility complex and T cell response (*12*) as well as the antigen's availability to regulate the immune response (*13*). Such responses are found in heterozygous populations and would need further study before proceeding with additional Eppin fertility trials, which might include a linear B cell epitope (fig. S7).

We tested the effect of Eppin immunization on male fertility at the Indian Institute of Science. Six adult male monkeys (*M. radiata*) were immunized with human recombinant Eppin and six controls received adjuvant only. High–anti-Eppin titers were detected in four of the six monkeys immunized with Eppin in squalene; two monkeys were low responders with titers <1:400 on postimmunization day

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Table 1. Fertility test of Eppin-immunized male monkeys (*M. radiata*). The seven males were 7 to 12 years old and had each sired 1 to 3 offspring in the previous 1 to 2 years. No pregnancies resulted from immunized males. Each exposed female had an estradiol 17β surge, indicating that an ovulation probably occurred in that cycle. ELISA O.D., enzyme-linked immunosorbent assay O.D. at 1:1000 on nearest day of cohabitation.

Male monkey no.	Cycle no.	Female monkey no.	ELISA O.D.	Days after first immunization
Group 1 Eppin (squalene)				
602				
	I	55	0.39	476
	II	88	1.19	522
	III	107	1.19	528
619				
	I	84	0.67	475
	II	64	0.56	523
	III	23	0.56	530
625				
	I	60	1.48	397
	II	97	1.00	525
	III	89	1.15	568
679				
	I	19	0.70	475
	II	47	0.62	534
	III	82	0.62	542
Group 2 Eppin (CFA)				
610				
	I	29	0.91	147
	II	73	0.59	225
	III	97	1.19	250
656				
	I	107	1.86	147
	II	47	1.93	225
	III	55	2.14	250
657				
	I	79	1.27	147
	II	6	1.53	276
	III	82	1.00	323

Table 2. Fertility test of male monkeys (*M. radiata*) in adjuvant control group. The six males were 10 to 11 years old and had each sired 1 to 3 offspring in the previous 1 to 3 years. Male 609 impregnated two different females. Four out of six males (67%) impregnated females.

Male monkey no.	Female monkey no.	Days after first immunization when conceived
569	37, 107, 47	No pregnancy
604	35	650
607	87	650
609	86; 6	502; 594
688	96	502
690	6, 84, 11	No pregnancy

126. Because the purpose of this study was to test the efficacy of antibodies to Eppin on fertility, the two low-titer monkeys were dropped from the study group and their fertility was not tested. Three additional males were added, which were immunized (primary immunization) with recombinant human Eppin in complete Freund's adjuvant (CFA) to boost immunogenicity. The original four monkeys immunized with Eppin in squalene were designated group 1, and the three additional males immunized with Eppin in CFA were designated group 2.

Antibody titers in all of the monkeys were >1:10,000 at the time the matings started and remained elevated throughout the mating period. Figure S4, A (group 1) and B (group 2), shows the mean optical density (O.D.) value at 450 nm for the monkeys at a 1:1000 dilution of serum. A titer of >1:1000 was sustained for 775 days in group 1 (fig. S4A) and for 481 days in group 2 (fig. S4B). There was no effect on serum testosterone levels in the immunized males in either group 1 or group 2 compared with control values (fig. S5) and no effect on sperm counts in either group (fig. S6).

Each male monkey in the immune and control groups was subjected to fertility testing by cohabiting with a proven fertile female between days 9 and 14 of her menstrual cycle. Each male was exposed to three ovulatory cycles of three different females to test their fertility. Immune and control groups began fertility testing on days 390 to 397 (Table 1 and table S2; for group 2, day 390 is 147 days after their first day of immunization). Group 1 completed testing on day 568, group 2 completed testing on day 566 (323 days after their first day of immunization), and the control group completed testing on day 691 (table S2). None of the immunized monkeys was able to impregnate females, indicating that males with sustained high–anti-Eppin titers were infertile (Table 1). Four monkeys in the adjuvant control group impregnated 5 females (4 out of 6, 67%, Table 2). All the monkeys used for breeding exhibited ovulatory cycles (table S1).

After the completion of fertility testing, immunizations of monkeys stopped on day 691 (day 448 of immunization for group 2). Groups 1 and 2 were maintained without further immunizations for 450 days (group 1; Eppin/squalene) and 451 days (group 2; Eppin/CFA), respectively, to test their ability to recover fertility after immunization. During this recovery time period, three of four monkeys in the Eppin/squalene group and two of three monkeys in the Eppin/CFA group recovered their fertility for a total recovery of 71% (5 out of 7; table S3). The males exhibited no symptoms of autoimmune disease and had no detectable serum titer of antibody to Eppin at 1:1000 dilutions.

This study demonstrates that effective and reversible male immunocontraception in primates is an attainable goal. We found that a high serum titer (>1:1000), sustained over several months, achieves an effective level of contraception. Seven out of nine males (78%) developed high titers to Eppin, and all these high-titer monkeys were infertile. Five out of seven (71%) high–anti-Eppin titer males recovered fertility when immunization was stopped.

Eppin on the surface of spermatozoa and in semen is bound to semenogelin (*14*), which is involved in coagulum formation in the ejaculate. We can speculate that one mechanism to explain the infertility is that antibodies to Eppin interfere with normal Eppin interaction with the sperm surface and with semenogelin.

References and Notes
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A Cluster of Metabolic Defects Caused by Mutation in a Mitochondrial tRNA

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Hypertension and dyslipidemia are risk factors for atherosclerosis and occur together more often than expected by chance. Although this clustering suggests shared causation, unifying factors remain unknown. We describe a large kindred with a syndrome including hypertension, hypercholesterolemia, and hypomagnesemia. Each phenotype is transmitted on the maternal lineage with a pattern indicating mitochondrial inheritance. Analysis of the mitochondrial genome of the maternal lineage identified a homoplasmic mutation substituting cytidine for uridine immediately 5′ to the mitochondrial transfer RNA^{Ile} anticodon. Uridine at this position is nearly invariate among transfer RNAs because of its role in stabilizing the anticodon loop. Given the known loss of mitochondrial function with aging, these findings may have implications for the common clustering of these metabolic disorders.

Hypertension and dyslipidemia are important risk factors for many common cardiovascular diseases, including myocardial infarction, stroke, and congestive heart failure (*1*, *2*).

These traits are concordant in individual patients more often than expected by chance (*3*, *4*). Large epidemiologic studies have demonstrated that subjects with hypertension