A Booster for Contraceptive Vaccines

Although the strategies can be controversial, advances in vaccines against pregnancy may make immunization a viable new approach to preventing unwanted reproduction

Nancy Alexander has a complaint about contraceptives, and as head of the contraceptive development branch of the U.S. National Institute of Child Health and Human Development (NICHD) in Bethesda, Maryland, she knows what she is talking about. "In the last 20 years," says Alexander, "we've had no [fundamentally] new type of contraceptive."

That's unfortunate, because current options in family planning are far from adequate. Despite the phenomenal success of the oral contraceptive pill, concerns linger about the safety of long-term use, particularly among smokers. Barrier methods such as condoms can have alarming failure rates under real-life "field" conditions, and intrauterine devices carry a small risk of permanent infertility due to pelvic inflammatory disease.

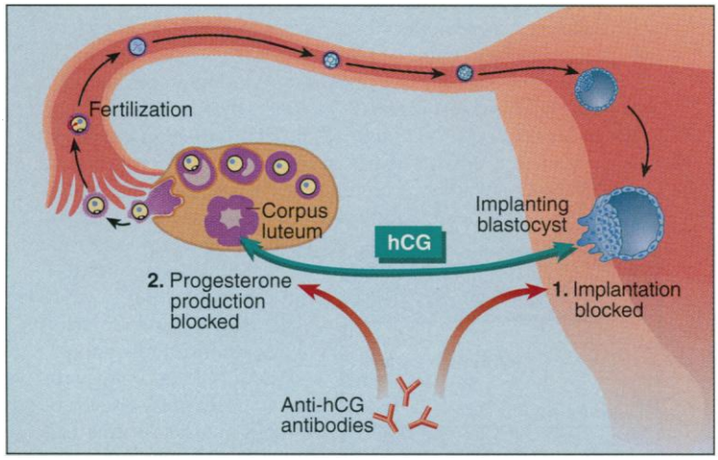
That is why contraceptive researchers are hailing as a landmark a paper in the 30 August issue of the *Proceedings of the National Academy of Sciences*, from a group led by G. P. Talwar of India's National Institute of Immunology in New Delhi. The paper provides the first demonstration that women can be vaccinated to prevent pregnancy. Talwar's team immunized women against human chorionic gonadotropin (hCG), a hormone produced by the ball of cells that develops from a fertilized egg and which is essential for implantation in the uterus. Four fifths of the vaccinated women produced large quantities of antibody to hCG, and the vaccine prevented pregnancy in nearly all of these women. "Bravo to Talwar," says gamete biologist John Herr of the University of Virginia, who is working to develop an anti-sperm contraceptive vaccine. "It's the result that says 'go.' "

This special issue on reproduction has news stories on contraceptive vaccines, the lagging development of other contraceptives, and attempts to forestall premature labor. Articles on implantation in mammalian pregnancy, sex determination in plants and mammals, the physiology of estrogen receptors, and other topics begin on page 1494.

The path to prevention

That "go" signal is sorely needed, say contraceptive vaccine researchers, who argue that their field, which shows great promise, has long suffered from sparse funding. Aside from Talwar's vaccine, they point out, one other anti-hCG contraceptive has entered human clinical efficacy trials. Further down the pipeline, other vaccines designed to keep sperm and egg apart are under development, and several are being tested in primates. Although most of these preparations are eventually intended for women's use, other researchers are focusing on vaccines designed to suppress sperm production or disable sperm before they are ejaculated (see box on p. 1485).

Yet a long path, strewn with obstacles, must be negotiated before any of these contraceptive vaccines reach the clinic. Concerns that anti-hCG vaccines may be opposed by pro-life groups on the grounds that they act after fertilization have kept many funding agencies out of the picture. And no vaccine that has yet reached primate trials matches the efficacy rate of oral contraceptives. David Griffin, who manages the contraceptive vaccine task force run by the World Health Organization's (WHO's) Geneva-based Special Program on Research and Training in Human Reproduction, puts it bluntly: "There's a long and difficult road from the lab bench to the buttock."



SOURCE: VERNON STEVENS

Immunizing against implantation. Antibodies to human chorionic gonadotropin (hCG) block the hormone, which helps implant the fertilized egg in the uterine wall. This affects the implanting blastocyst, as well as the corpus luteum.

Talwar and WHO have been traveling down that road for two decades, developing different anti-hCG vaccines. That hormone offers a good target, explains Griffin, because it is produced in a very specific set of circumstances: by the developing embryo, beginning before implantation. That's desirable, says Griffin, because a vaccine that provokes an immune response against a normal body constituent would run the risk of inducing a severe autoimmune reaction.

Both preparations trick the immune system into producing antibodies against hCG by using one part of the molecule: the beta-hCG subunit. Because the beta subunit is not normally recognized as a "foreign" molecule by the immune system, the vaccines prompt an immune reaction by attaching the beta sequence to a toxoid—a toxin produced by a disease-causing bacterium. The toxin has been chemically altered to render it harmless, but it still induces a strong immune response.

Researchers were unable to use the entire hCG molecule in the vaccine because the beta subunit's partner, the alpha subunit, is identical to a sequence carried by two other hormones produced by the pituitary gland; if the alpha subunit were incorporated into the vaccine, it might provoke an autoimmune response, explains Vernon Stevens of Ohio State University, in whose lab the WHO vaccine was developed. To further reduce the chance of pituitary damage, WHO's candidate anti-hCG vaccine is based on only a fragment of the beta subunit, which is not present in pituitary hormones: a 37–amino acid sequence located at one end. WHO's vaccine is now entering clinical efficacy trials in Sweden.

Talwar, in contrast, has long argued that WHO's approach is overly cautious, and his vaccine contains beta-hCG in its entirety. That might seem risky, but the evidence so far seems to vindicate Talwar. Long-term toxicology studies in monkeys and baboons have shown no autoimmune pathology. And women injected with the vaccine in safety trials in 1986 have shown no ill effects.

Both anti-hCG vaccines, however, share

Searching for a Vaccine for Men

In contraception as in many other facets of modern life, true gender equality is still some ways off. Women worldwide take contraceptive pills, get fitted with intrauterine devices, and use a range of barrier methods to prevent pregnancy. But when it comes to male contraceptives, it's the good old-fashioned condom ... or nothing. "Men don't have any long-acting reversible methods," says biochemist Rosemarie Thau, director of contraceptive development at the Population Council in New York City.

Thau, however, is one of a handful of contraceptive vaccine developers who aim to correct this sorry state of affairs. In men, the focus is on reproductive hormones and sperm, and two vaccines have reached the stage of safety trials in human volunteers.

Thau's vaccine induces men to make antibodies against gonadotropin-releasing hormone (GnRH), secreted by the pituitary gland, which stimulates the production of testosterone and sperm. The preparation is being tested for safety in patients suffering from prostate tumors, which are stimulated to grow by testosterone. Thau also wants to test the vaccine as a contraceptive, but other researchers caution that it has a downside: Men treated with the vaccine lose their libido, unless they are given hormone replacement therapy. That may be an acceptable side effect for a cancer treatment, but probably not for a contraceptive.

Immunization equality. Biochemist Rosemarie Thau is developing a vaccine against a hormone that stimulates sperm production.

That's why some researchers are more enthusiastic about the approach of a group led by Raghuveer Moudgal of the Indian Institute of Science in Bangalore, which is targeting follicle-stimulating hormone (FSH). Like GnRH, FSH is required for sperm production, but it doesn't affect the release of testosterone. Moudgal has shown that in monkeys, immunization with sheep FSH induces a relatively long-lasting, but reversible, period of infertility. Safety trials in human volunteers, moreover, have been conducted without major incident. "What we have to prove right now is the efficacy [in men]," says Moudgal.

Despite Moudgal's promising results, however, many researchers remain uneasy about the idea of using the immune system to attack hormones that are produced by the body at all times and which circulate freely in the blood; they still fear this is a recipe for dangerous side effects. A better approach, say some scientists, would be to target antigens carried by sperm cells.

Significantly, in 1988 Paul Primakoff and Diana Myles of the University of Connecticut showed that immunization with the sperm surface protein PH-20 induced infertility in male guinea pigs as well as females (see main text). PH-20, unfortunately, also causes inflammation of the testes due to an autoimmune reaction. But Primakoff and Myles are now working on a second sperm antigen, dubbed fertilin, which undergoes a subtle chemical change after sperm leave the testes but before they are ejaculated. That means that it should be possible to get the immune system to attack the molecule in its final form only, removing the danger of autoimmune testicular damage. And if approaches like that are successful, it could begin to bring equality between men and women one step closer—at least in the field of contraception.

–P.A.

one major drawback: They stimulate the production of large quantities of antibodies for only a few months, and so require regular booster injections to maintain antibody production. The irony is that when contraceptive vaccines were first proposed, many researchers feared the effect would be permanent, rendering the strategy useless for producing a reversible contraceptive. It's now clear that those fears were unfounded, and the problem is just the opposite: to induce infertility over a sufficiently long period—say a year or more with one injection—to make the vaccines a viable alternative to existing contraceptive methods. "It won't be very attractive if they have to come in frequently to get shots," says Stevens. To get around that problem, both he and Talwar are experimenting with putting the vaccines into microscopic biodegradable polymer spheres. The idea is that when injected into muscle, these spheres will slowly release the contraceptive as they degrade.

Whether or not Talwar and Stevens are successful in inducing long-lasting infertility, researchers say it's important to persevere with alternative approaches—particularly given the likely moral objections to any method that exerts its effect after fertilization. Talwar and Stevens reject any suggestion that their vaccines induce abortions. There's clear evidence that anti-hCG vaccines act before implantation, says Talwar, as women treated with his vaccine don't have elongated menstrual cycles: If the vaccine expelled the embryo shortly after it implanted, each cycle that resulted in fertilization would be lengthened by the amount of time the embryo had spent attached to the uterine wall. But while the medical definition rules that pregnancy begins only after implantation, many lay people believe the key moment is fertilization—a position that guides the policy of U.S. federal agencies not to support anti-hCG vaccine development. "Our Congress would think of it as an abortifacient," says NICHD's Alexander.

The politically correct target

Even some ardent pro-lifers, however, might be willing to countenance a vaccine that prevents sperm and egg from fusing. "The event to go for is fertilization," argues gamete biologist John Aitken of the U.K. Medical Research Council's Reproductive Biology Unit in Edinburgh. In practical terms, that means targeting either the zona pellucida—the transparent layer of glycoproteins that surrounds the egg cell—or antigens carried by sperm. The goal: to coat egg or sperm with antibodies, so that the two gametes are unable to fuse.

The zona approach received a major boost in 1989, when a team led by Jurrien Dean of the U.S. National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda reported in *Science* that pregnancy could be blocked in female mice by vaccinating them with a small synthetic peptide containing a seven–amino acid sequence from a molecule called ZP3, one of the three glycoproteins that make up the mouse zona pellucida. The peptide was attached to an immunogenic "carrier" molecule, whose function, like that of a toxoid, is to provoke a response against a molecule that ordinarily is not attacked by the immune system.

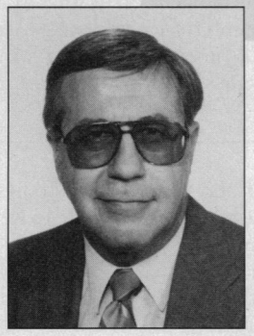
Yet despite strenuous efforts over the last 5 years by several groups, including Aitken's team, the same approach has proved unsuccessful in primates, which are the most appropriate testing ground for human contraceptives. Aitken's group has recently obtained positive results in monkeys using intact ZP3. But there is a serious drawback: severe autoimmune reactions that destroy immature eggs in the ovaries—clearly an unacceptable side effect for a human contraceptive. "I'm not going to put myself through a premature menopause," observes Bonnie

Dunbar, an anti-zona vaccine developer at Baylor College of Medicine in Houston. Several researchers are now trying to identify small peptides that exert a contraceptive effect, but do not provoke an autoimmune reaction.

Many researchers, however, argue that a better way of avoiding autoimmune pathology would be to produce a vaccine for women based on antigens from sperm, which will be foreign to females. And there's good reason to think it should be possible to produce a safe and effective anti-sperm vaccine: A small proportion of infertility in both women and men seems to be due to the production of anti-sperm antibodies.

The strategy poses a formidable technical hurdle, however. There are millions of sperm in a single ejaculate, and to hit this large and moving target, researchers must ensure that antibodies are secreted into the female reproductive tract by its mucosal lining. Despite recent progress by vaccinologists in stimulating this "mucosal immunity" (*Science*, 9 September, p. 1522), researchers are still some ways from being able to manipulate such responses at will.

But there are signs that this hurdle will not be insurmountable. In a 1988 paper in *Nature*, a group led by the husband-and-wife team of Paul Primakoff and Diana Myles of the University of Connecticut showed a 100% contraceptive effect in female guinea pigs immunized with a sperm protein called PH-20. The effect was reversible, and there was no need to couple PH-20 to an immunogenic molecule, as the protein is readily recognized as foreign by the female immune system. Since then, the Connecticut researchers have concentrated on cloning the PH-20 genes from a range of species including monkeys and humans and working out how to make large quantities of species-specific PH-20 using recombinant DNA technology. Now, they're ready for the next big test: repeating their guinea pig experiments in primates.



Getting a better response

To date, only one candidate anti-sperm molecule has been shown to depress fertility in primates. This is a form of the enzyme lactate dehydrogenase (LDH) that seems to be unique to sperm. It was discovered three decades ago by biochemist Erwin Goldberg of Northwestern University in Evanston, Illinois. In an experiment due to be published next year in *Biology of Reproduction*, Goldberg found that immunization with the sperm-specific LDH caused a 78% reduction in fertility in a group of female baboons, compared with unvaccinated controls. When the animals were "rested" for a year, says Goldberg, their fertility returned to normal.

That's an encouraging result, but the efficacy of Goldberg's vaccine remains far below that of existing contraceptives. "For a vaccine to be viable," says Primakoff, "you have to get it above 95% efficacy." With the notable exception of Primakoff's guinea pig experiments, however, contraceptive vaccines have struggled to meet that requirement. Indeed, even Talwar's vaccine does no good for the 20% of women who fail to produce large amounts of anti-hCG antibodies following immunization.

Contraceptive vaccine developers, however, are confident that response rates will be improved. The problem is thought to lie with the activation of immune system cells called "helper" T cells, which provoke the immune system's antibody factories, B cells, into action. In an immune response, foreign antigens first become bound to glycoproteins encoded by genes in the major histocompatibility complex (MHC), which are carried by so-called antigen presenting cells. Helper cells can then recognize these antigens and stimulate antibody production. However, each MHC glycoprotein binds to a different range of antigens. So given natural genetic variation in the MHC, women who respond poorly to Talwar's vaccine are probably simply producing a range of MHC glycoproteins that fail to bind effectively to it.

"[Contraceptive vaccines] won't be very attractive if [people] have to come in frequently to get shots."

—Vernon Stevens

If so, Talwar says it should be possible to boost the response rate by replacing the toxoid carrier used to stimulate helper T cells with a cocktail of "promiscuous T cell epitopes"—peptides that bind to a wide range of different MHC glycoproteins. "The eventual aim is to have close to 100% of women responding," he says.

Nevertheless, refining even Talwar's vaccine into a usable product will take time, and he warns against expecting the widespread introduction of an anti-hCG vaccine before the end of the decade. But as WHO's Griffin points out: "The need for new methods is going to be as great—if not greater—in 10 years as it is now."

–Peter Aldhous

Peter Aldhous is a writer at New Scientist.

PERINATAL RESEARCH

Stopping Premature Births Before It's Too Late

Too much, too soon, is one way to describe the current situation with premature births. "A national disgrace," is how Robert Garfield, director of Reproductive Sciences at the University of Texas Medical Branch in Galveston, refers to it. Figures compiled by the Centers for Disease Control show that in the United States more than 10% of babies are born prematurely. According to a 1985 National Academy of Sciences report, some $5 billion is spent on premature infants, mostly for high-tech neonatal care. However, the money is no guarantee of survival: A 1985 study published in the *British Medical Journal* estimates that premature births account for 85% of all early infant deaths. And the babies who live are prone to a host of problems, ranging from blindness and cerebral palsy to poor school performance. "The problem is just devastating, especially to parents," says Garfield. But now there are some signs that help is on the horizon.

Traditional efforts to prevent premature births have focused—with limited effectiveness—on stopping labor contractions that have already begun. So researchers are developing a new paradigm, trying to pinpoint the causes of premature labor in hope of nipping the problem in the bud. "The real issue," says perinatal researcher and infectious-disease specialist James McGregor of the University of Colorado Health Sciences Center in Denver, "is to prevent premature birth and avoid the whole issue entirely."

Much of this work focuses on identifying and treating uterine infections, which some researchers consider to be a major cause of premature births. In other efforts, scientists are looking for biochemical markers that signal that labor is about to begin. A clinical trial of the efficacy of using one such marker has already started. And finally, scientists are taking a new look at the physiology of uterine contractions, hoping to find ways to prevent them if they begin prematurely. "I'm very excited about the possibilities," says parturition researcher Charles Lockwood of the Mount Sinai School of Medicine in Manhattan. "We're attacking the problem with a vengeance from several standpoints."

Such a wholesale effort may be necessary, because the causes of premature birth are many and varied, ranging from inadequate blood flow to developmental disorders to allergies.