ACC&D
Alliance for Contraception in CATS & DOGS

Immunocontraceptive Approaches for Sterilization of Dogs and Cats

Scientific Think Tank
November 19-21, 2009
Roanoke, VA

Overview

On November 19-21, 2009, a think tank on the use of immunocontraceptive approaches to sterilize cats and dogs was held at the Hyatt Place hotel in Roanoke, Virginia. The meeting was convened by the Alliance for Contraception in Cats and Dogs (ACC&D), with support from the Found Animals Foundation (FAF) and the Animal Assistance Foundation (AAF), to discuss the potential of using immunization approaches in the pursuit of a non-surgical sterilant for cats and dogs.

The mission of the ACC&D is to expedite the successful introduction of non-surgical methods to sterilize cats and dogs and to support the distribution and promotion of these products. ACC&D's motivation is to reduce animal death and suffering worldwide by enhancing the tools available to humane population control programs. Non-surgical approaches are presumed to be less expensive and less labor-intensive options for sterilization, allowing far more animals to be treated quickly and safely.

Immunocontraception was chosen by the ACC&D board and scientific advisors as one of two research areas that present the most promise for achieving the goal of a non-surgical sterilant.

A mature area of research, immunocontraception has met obstacles in its application to cats and dogs, but recent scientific advances may provide the opportunity for breakthroughs in this regard.

Dr. Levy and her research cats

The think tank would address specific challenges experienced to date in the application of immunocontraception, including generating a sufficiently long duration of effect with one treatment, inducing a high (95%+) response level in treated animals, and producing few or manageable negative side effects.

Joyce Briggs described the hoped-for outcomes of the think tank to include conclusions and recommendations for future research in this area of exploration. These will be presented at the ACC&D Symposium to be held in Dallas, Texas, in April 2010.

Attendees

(See www.acc-d.org for bios of participants.)

Foundation and Nonprofit Representatives:

Joyce Briggs, MS	President, Alliance for Contraception in Cats and Dogs, Portland, Oregon
Shirley Johnston, DVM, PhD	Director of Scientific Research, Found Animals Foundation, Los Angeles, California

Scientific Panel:

Note: An asterisk (*) indicates the two chairmen of the think tank.

Harini Bagavant, MBBS, PhD	Assistant Professor, Department of Medicine, Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, Virginia School of Medicine, Charlottesville, Virginia
Stephen Boyle, PhD *	Professor of Microbiology, Director of the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
David A. Brake, PhD	Founder and Principal, BioQuest Associates, LLC
Scott Coonrod, PhD *	Associate Professor of Reproductive Biology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York
Roy Curtiss III, PhD	Director, Center for Infectious Diseases and Vaccinology, Biodesign Institute, Professor, School of Life Sciences, College of Liberal Arts and Sciences, Arizona State University, Tempe, Arizona
Gregg Dean, DVM, PhD	Professor and Director, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina
Julie Levy, DVM, PhD, DACVIM	Director, Maddie's Shelter Medicine Program, College of Veterinary Medicine, University of Florida, Gainesville, Florida
Colin R. Parrish, PhD	John M. Olin Professor of Virology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York
Beverly Purswell, DVM, PhD	Professor of Theriogenology, Department of Large Animal Clinic Sciences, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
Paul Christopher Roberts, PhD	Associate Professor of Virology, Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
John T. Schiller, PhD	Senior Investigator, National Cancer Institute, NIH, Bethesda, Maryland

Topics of Discussion

Overview of the Challenge

Joyce Briggs introduced the purpose of the think tank and explained ACC&D's interest in promoting the discovery and introduction of non-surgical alternatives for the sterilization of cats and dogs. Both she and Julie Levy presented background data on dog and cat overpopulation and on challenges presented by reliance on surgical sterilization to control these populations. Much of this background information is omitted from this summary, but is readily available through other sources, including ACC&D's website (www.acc-d.org).

ACC&D is interested in finding safe and effective alternatives to surgical sterilization for animal populations with limited or no access to veterinary care. Alternatives to surgery could become a key tool for agencies treating homeless populations of cats and dogs seeking adoptive homes, or feral cats through trap, neuter, release (TNR) programs. Other key U.S. markets for non-surgical alternatives include low-income families for whom surgical sterilization is a financial burden, and pet owners opposed to surgery but who do not object to sterilization of their pet.

Worldwide, different issues dominate the subject of cat and dog overpopulation than in the U.S.; free-roaming dog populations present a public health risk in many parts of the world, where dog bites are an important source of human rabies cases. Controlling the owned, community and stray dog populations is difficult in many countries where low rates of surgically sterilized dogs result from a lack of trained small-animal veterinarians and insufficient funding to support their work, compounded by the large number of animals to be treated.

Julie Levy outlined the advantages of sterilization surgery, including results that are predictable, immediate, permanent, and have known behavioral and medical benefits. Disadvantages of surgery include that it is invasive, has a low but extant rate of morbidity and mortality, is expensive, and requires anesthesia and recovery time. Ideally, alternative treatments would be effective with a single dose, provide permanent sterility and be cost-effective. For most markets, it is preferable for non-surgical contraceptive or sterilization treatments to suppress female estrus cycles, and hormone-based behaviors such as spraying for male cats and marking for male dogs. These are judged to be behavioral challenges to pet keeping and contribute to nuisance behavior in free-roaming animals. The veterinary field in many parts of the world also strongly supports surgical sterilization of female dogs and cats to reduce risks of mammary cancer and reproductive health issues such as pyometra, and wants to retain that benefit in methods of birth control.[1]

Julie Levy described some of the current efforts to control feral cat and dog populations worldwide through surgical sterilization. A study carried out in Jaipur (1) over eight years, in which nearly 25,000 dogs were trapped, vaccinated for rabies, surgically spayed or neutered and re-released, observed a 28% decrease in the dog population while human rabies cases fell to zero, providing dramatic evidence of the ability to combine efforts to improve human health alongside animal welfare. Operation Catnip, a feral cat spay/neuter program founded by Julie Levy, can carry out 250 operations in a day and has sterilized 40,000 cats since 1994. Despite this immense effort, cats reproduce quickly and populations remain large; only about 2% of trapped feral and community-owned cats are neutered (as opposed to an 85% sterilization rate for pet cats) and therefore this population is largely responsible for the cat overpopulation problem in the U.S. Julie Levy expressed the opinion that a non-surgical alternative would be extremely helpful in treating feral and community-owned cats.

[1] ACC&D advisor Steve Zawistowski, PhD, CAAB, notes the complexity of the issues surrounding non-reproductive effects of surgical sterilization and hormone suppression on health and behavior. For more information, see the entire session *Non-reproductive Effects of Spaying and Neutering*, ACC&D's Third International Symposium Proceedings, 2006, or *Benchmarking Surgical Sterilization: A Review of the Safety, Efficacy, and Non-reproductive Effects of Surgical Sterilization, as a Benchmark for Assessing Non-surgical Approaches,* I Reichler, ACC&D's Fourth International Symposium Proceedings, 2010.

Shirley Johnston of Found Animals Foundation (FAF) provided an overview of the Michelson Grants in Reproductive Biology, which has pledged to fund up to $50 million in research in the pursuit of a "safe, effective, and practical non-surgical sterilant for use in cats and dogs." The Michelson Prize in Reproductive Biology will award $25 million to the first entity to provide a safe, effective and practical non-surgical sterilant that works in cats and dogs, both males and females. The Michelson Prize and Grants Program offers the most research funding ever available to spur research in this field, as funding for control of reproduction in cats and dogs has been generally unavailable from major comparative biomedical research funding agencies.

The panel discussed the importance of defined goals for research awards, and John Schiller and Colin Parrish noted that the goal of the Michelson Prize might be a disincentive to researchers who find it unrealistic to achieve a single treatment, resulting in a permanent effect in cats and dogs, males and females, in the short term. The panel emphasized the importance of achieving stepwise or incremental goals for encouraging participation and defining success of the program.

For example, studies have estimated the average life span of both feral cats in the U.S. and spayed community dogs in India at not much more than three years, indicating that a contraceptive effective for at least three years could have a significant effect on population numbers (2). The panel proposed that such a contraceptive, effective for at least three years, might be an appropriate interim goal. John Schiller mentioned incremental goals for cost might also be desirable, since a product can be sold now to those who can afford it and cost may come down as contraceptive use increases and efficiencies in manufacture are realized. Human Hepatitis B vaccine was given as an example: in 20 years the price has fallen from $80 to $0.18 a dose. Colin Parrish also suggested incremental effectiveness goals could suffice, depending on the target population; though 100% effectiveness of a fertility control treatment may be expected by owners of companion animals, 80% effectiveness might still be helpful in the control of feral populations. David Brake also proposed that an approach that requires more than one treatment (e.g., an initial vaccination and a later booster) might be an incremental goal, useful for owned animals, and once efficacy is demonstrated, a one-treatment technology could be developed.

Canine and Feline Reproductive Biology

Beverly Purswell presented a review of idiosyncrasies of canine and feline reproduction as background material.

Theriogenologists Dr. Purswell and Dr. Johnston confer at the think tank.

Unique aspects of dog reproduction include the fact that diestrus (progesterone phase) occurs after every estrus; if not impregnated, bitches will experience a pseudo-pregnancy lasting the same length of time as an actual pregnancy, unlike most animals which experience a decrease in progesterone as soon as it is determined that the animal is not pregnant. Dogs also ovulate primary oocytes requiring two meiotic divisions before fertilization, and canine follicles undergo pre-ovulatory luteinization. Dogs are monestrus, meaning they have one estrus cycle per season, which usually averages one to three cycles per year for domesticated dogs.

In contrast, domestic cats are induced ovulators, stimulated to ovulate in response to mating, though spontaneous ovulation events have been observed. Cats are prolific breeders, able to become pregnant within days after delivering a litter, and have been demonstrated capable of producing five litters in one year. Additionally, a cat can become sexually mature as early as 4 to 5 months of age. Cats are also seasonal breeders, induced to reproduce by long daylight hours, such that equatorial populations breed year-round.

The panel discussed the ideal time to administer a contraceptive treatment. Though prepubertal would be desirable, the fact that the treatment is aimed at shelter and feral populations requires that the treatment not be age or cycle dependent. The panel agreed that targeting females rather than males was most important to achieve population control.

Brief Overview of Immunology Terminology and Cell Types

The immune system protects the body against infection and removes abnormal cells. Two broad types of immune responses can be described: humoral immunity, which is mediated by antibodies secreted by plasma (B) cells in response to extracellular antigens, and cell-mediated immunity, in which cytotoxic T cells, natural killer cells and macrophages are activated in response to intracellular antigens, such as virus-infected cells. In some cases, the immune system can be activated inappropriately, resulting in inflammation in response to an antigen that is not actually dangerous (i.e., allergic reactions), or in an attack on normal "self" tissue (auto-immune disease).

Two immune cell types important to the discussion of immunization are B cells (lymphocytes that mature in the bone marrow) and T cells (lymphocytes that mature in the thymus). Both of these cell types recognize antigens – either pathogens, non-self peptides, or peptides not previously subjected to surveillance by the immune system for distinction between self and non-self antigens – causing the cells to become activated. Activated B cells bind free antigen, and can be stimulated by helper T cells to proliferate and secrete their antigen binding receptor. These secreted antibodies bind to antigen, targeting pathogen for destruction via phagocytosis, or neutralizing the activity of peptides by interfering with their binding to targets. B cells can also be activated in a T cell independent manner in response to a limited number of antigens, including repeating carbohydrate epitopes on bacterial cell walls.

Several classes of T cells were discussed during the think tank, including helper T cells (CD4+ T cells), regulatory T cells (CD4+CD25+foxp3+ T cells, also called Treg cells) and cytotoxic T cells (CD8+ T cells). Treg cells provide an important control on the immune system, preventing immune responses from becoming too strong, and also protecting against autoimmunity. In some cases, initiating an immune response against a non-foreign (self) protein might require overcoming immune tolerance (the ability of the immune system to recognize and not mount a response against self antigens) by reducing the protective effect of Treg cells. Tolerance generally occurs during fetal development, when developing lymphocytes that display affinity for self antigens are deleted.

Immunocontraception might involve stimulation of both humoral and cell-mediated arms of the immune system, with the goal of producing antibodies to inactivate molecules important for fertility, and/or activating the cell-mediated response due to cytotoxic T lymphocytes to specifically destroy cells required for fertility. The panel was interested in whether an effective immune response could be mounted against antigens in the reproductive system, i.e., whether these tissues are subject to immune tolerance, since some antigens might appear only once reproductive maturity is reached and not be subjected to selection.

Dr. Parrish leading the discussion

At the conclusion of the think tank, Colin Parrish consulted with Alex Travis and Doug Antczak of Cornell University, and provided the following summary of what is known about this issue.

The regulation of immune responses to antigens associated with male or female reproductive tissues is

complicated; some cells and tissues develop at the time of sexual maturation, long after the differentiation between self and non-self that occurs early in development. Therefore, these tissues would normally be considered new antigens, yet there are few or no immune responses mounted against most of these tissues. Multiple mechanisms have been described to explain this observation, including mechanical sequestration of the tissues that prevents exposure of the antigens to the immune cells; active immune regulation by various regulatory lymphoid cells, including Th17 T cells and IL10 secreting cells (and likely others); secretion of immunosuppressive hormones; low expression of the antigens; and active or passive tolerance. In most cases, more than one of these mechanisms may act in combination.

In the male, a variety of antigens develop at the time of sexual maturation, including antigens on meiotic, post-meiotic and morphologically mature sperm. A "blood-testis barrier" exists between Sertoli cells, which reduces exposure of testis antigens to the general blood and lymph circulation; however, new data are showing that this is not a complete barrier to antigens. Therefore, additional regulatory mechanisms, such as those listed above, likely suppress immune responses to antigens in the testis and epididymis.

In the female, the primary targets for immunocontraception are the germ cells of the ovary, which develop before birth. In the female there are fewer mechanical barriers to the immune system, so any immune protection would normally come from combinations of the immune suppression and tolerance mechanisms listed above.

It is well established that molecules of the zona pellucida (ZP) that surrounds the early developing embryo can be highly antigenic when presented to the maternal immune system in an appropriate manner. Immune responses to such antigens have been shown to suppress fertility in several species (seals, horses) and the mechanism may be either a 'coating' of the oocyte with immune complexes, preventing fertilization, or interaction of antibodies with fertilized oocytes.

There are also significant differences between species in the way in which these potential anti-self responses are regulated, and very little research has been conducted on companion animals. Thus, the specific immune response to a "self" antigen that might be useful for immunocontraception in the cat and in the dog would need to be determined.

Research to Date into Immunocontraception and Population Control in Cats and Dogs

Julie Levy presented a brief review of studies attempting immunocontraception in cats and dogs. SpayVac™ is a wildlife vaccine that is not yet approved, containing porcine zona pellucida (PZP) proteins as antigen, packaged in liposomes, and formulated in Freund's Complete Adjuvant (FCA). When injected, the resulting antibodies bind to native ZP protein on the exterior of the ovum, blocking sperm binding sites and preventing fertilization. SpayVac™ has been shown to work well in seals, providing up to six years of contraception with one dose, and has been used to control deer populations and several other wildlife species. However, when similar experimental vaccines were tried in cats using either the original porcine ZP formulation or ZP proteins prepared from the ovaries of cows, cats, ferrets, dogs or mink, all cats responded by producing anti-ZP antibodies but the antibodies did not bind the ovaries and fertility was not impaired (3, 4). Cats did produce antibodies that bound to the ovary when DNA plasmid vector vaccines expressing feline ZPA or ZPB+C were used (5), indicating that there may be some potential for the approach to work in cats. In dogs, studies have observed a stronger immune response to porcine ZP than to recombinant hZP3, and severe injection-site reactions with mFCA that were avoided by using CpG-DNA as adjuvant (6). The majority of dogs treated with four injections of recombinant ZP3 with CpG-DNA remained fertile in a follow-up study, indicating that improved antigenicity of the recombinant protein will be required

to inhibit fertility (6). One study has found that immunization of bitches with recombinant ZP3, conjugated to diphtheria toxin to increase antigenicity, resulted in reduced fertility (7). Roy Curtiss emphasized the importance that species-specific glycosylation of the ZP proteins might play in the induction of functional antibodies against ZP.

Roy Curtiss questioned whether the SpayVac vaccine works in the way the designers expected, by blocking ZP function, or if the ovaries are destroyed by an autoimmune response. Harini Bagavant mentioned that massive inflammation alone does not affect fertility but antibody specificity is required. Stephen Boyle added that some wild Assateague horses developed ovarian dysfunction five to six years after annual immunization with porcine ZP.

GonaCon™ is another immunocontraceptive developed for wildlife population control, consisting of synthetic GnRH (gonadotropin releasing hormone) bound to KLH (keyhole limpet hemocyanin) and an adjuvant called AdjuVac™, which is a diluted version of Johnes disease vaccine (Mycopar™- Ft. Dodge) containing killed mycobacterium and oil. The vaccine is designed to work with a single treatment, and has been studied in squirrels, swine, wild horses, white-tailed deer and other species. In swine and deer, infertility has lasted up to five years after a single treatment. In one study of white-tailed deer, a single injection of GonaCon was found to be 88% effective the first year and 47% effective the second year. Preliminarily, GonaCon has been found to induce GnRH antibodies in male cats (8). When female cats were given a single injection, pregnancy was prevented for 87% of cats for one year, 67% of cats for two years, 54% of cats for three years, 33% of cats for four years, and 27% of cats for five years. However, 24 months after vaccination, 6/20 cats had palpable non-painful injection site granulomas. It was discussed that treated male dogs suffered more severe adverse injection site reactions (relatively soon after injection) with painful draining tracts resulting at the injection sites. The panel discussed this vaccine's design being first-generation technology in the vaccinology field, and that much better preparations should be possible to avoid side effects.

A third interesting product is a GnRH vaccine produced by Pfizer, and conditionally licensed for use by the USDA, for treatment of benign prostate hyperplasia (BPH) in dogs, Canine Gonadotropin Releasing Factor Immunotherapeutic. This BPH vaccine is not labeled for use as a contraceptive, and may be discontinued by Pfizer, perhaps because of the small market of intact aged male dogs and availability of alternative treatments for BPH. This vaccine requires an initial injection and a booster after one month, followed by boosters every six months.

It was noted that additional positive side effects of immunization against GnRH might increase the market for a GnRH vaccine; after surgical sterilization, GnRH levels in bitches, for example, increase and may contribute to post-spay side effects such as incontinence. Immunization might prevent this problem in older spayed female dogs.

To reach the most feral cats, Julie Levy stated that an oral delivery via bait in the environment would be the most efficient distribution method, but noted the concern that non-target animals, such as endangered species or humans, could be inadvertently affected. Because of this, environmental delivery faces serious regulatory hurdles.

Julie Levy also presented a brief overview of the tissues and signaling pathways important to cat and dog reproduction, including the hypothalamus, which secretes gonadotropin releasing hormone (GnRH) to the pituitary which in response secretes the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH), which act on the reproductive organs of both males and females. Feedback inhibition from the sex hormones estrogen, testosterone and progesterone reduces GnRH levels, completing a negative feedback loop which controls reproduction.

Potential Molecular Targets

The panel compiled a list of potential molecular targets for immunocontraception, including GnRH, gonadotro-

pins (LH and FSH), gonadotropin receptors, and germ-line or reproductive-organ-specific proteins.[2]

Great interest was expressed in targeting GnRH, since immunization against GnRH has the potential to work in cats and dogs, and both males and females. Reducing GnRH levels long-term appears to be safe based on the existing literature. Concerns regarding GnRH as a target include the fact that it is a small 10 amino acid peptide and not immunogenic unless coupled with a large hapten, and is tolerated by the immune system as a self-protein. However, means to break tolerance were discussed (see below).

Another concern regarding GnRH is that it is highly conserved, and that vaccines will impact species other than cat and dog if exposure (e.g., in the environment) were to occur. For example, a baiting approach would be best to reach free-roaming or feral animals, but it would be difficult to restrict access to only the desired species. Even if it were possible to incorporate a species-specific virus or delivery system, there would be concerns about specific targeting to domestic felids and canids without affecting closely related wild populations such as the Florida panther, coyotes and wolves. The panel also discussed the dangers of producing an effective vaccine inducing permanent sterility, in that unethical or immoral individuals could use such an agent on other populations, including humans.

Targeting LH or FSH receptors did not generate much support, since there are many tissues outside the reproductive system that contain receptors for these molecules and that might be adversely affected. Targeting GnRH receptors per se may also produce side effects, in that GnRH receptors have been shown to exist in tissues other than the pituitary, and their role is being actively investigated[3]. However, reduction in GnRH levels by generation of anti-GnRH antibodies has been demonstrated to be safe and not associated with any undesirable side effects. Primordial follicles of the dog do not express the FSH receptor, so this is not an effective target in the dog. Also, John Schiller explained that it is more difficult to break tolerance and raise an immune response to a membrane protein such as a receptor. For all of these reasons, the panel concluded that the best options are to go "higher up" in the reproductive endocrine pathway by targeting GnRH, or to ablate the gonad.

Germ-line or reproductive-tissue-specific targets were discussed as a way to direct an immune response to destroy an essential cell population, resulting in sterilization. Alternatively, antibodies might be able to block binding sites, as in the ZP vaccines in which antibody binding interferes with fertilization. Harini Bagavant described an experimental model, in which a percentage of mice thymectomized at day 3 lose self/non-self differentiation ability and develop autoimmune disease, including ovarian failure. Scott Coonrod described a strong candidate antigen identified using this model. The protein, called "Maternal antigen that embryos require" (MATER), is cytoplasmic, is present in primordial follicles, and is expressed only in the oocyte.

Drs. Bagavant, Dean and Robert at the think tank.

Harini Bagavant also presented results of using ZP3 peptides in adjuvant to immunize mice; antibodies were generated, the number of which correlated with infertility, but a concomitant increase in inflammation also occurred, making it difficult to separate whether the antibody or inflammatory response or both were responsible for the ovarian pathology. In this model, eggs are destroyed, but primordial follicles remain so new eggs continue to mature. For this reason, ZP has not been seen as a perfect target when the goal is permanent sterilization. However, Harini Bagavant pointed out that earlier studies using ZP3 were designed to achieve a

[2] ACC&D scientific advisors note that this document is a summary of a single discussion at a point in time, and does not constitute a comprehensive review of the literature or of research related to immunocontraception. Other targets are of interest, including hypothalamic peptides that control GnRH such as kisspeptin and GnIH. (See *Endogenous GnRH Control: Kisspeptin and GnIF*, D Fellmann, C Pralong and PY Risold, ACC&D Third International Symposium Proceedings, pp 51-52, 2006.)

[3] Skinner, DC *et.al*. (2009) *J. Neuroendocrinology* 21:282-292.

human contraceptive as opposed to a permanent sterilant, and therefore specifically aimed to protect the primordial follicle. Study of other ZP proteins and identification and study of immunogenic ZP peptides can provide future avenues for research into achieving permanent sterility via targeting of ZP. Most ZP vaccines have used ZP proteins extracted from tissue, but more recent vaccines incorporate recombinant protein that is cheaper and easier to produce. ZP is highly glycosylated, and the fact that recombinant proteins expressed in bacteria will be missing this post-translational modification and therefore have reduced antigenicity was discussed; it was noted that yeast can be engineered to produce glycosylated recombinant proteins, though this glycosylation may not be identical to that which occurs in cats or dogs.

Gene expression is well described in various stages of mouse oocytes and Gregg Dean reported a published study characterizing gene knockouts associated with male infertility in mice. Some of the identified genes should be well conserved across species and a bioinformatics approach could identify similar genes expressed in cat or dog reproductive tissues. Chris Roberts suggested phage libraries to identify new or novel antigens. There was emphasis that the think tank should not make specific recommendations that could be seen as limitations, but rather allow scientists to identify their own targets of interest.

Roy Curtiss emphasized the importance of confirming that expression of a target is restricted to the tissue of interest, to avoid initiating an autoimmune response that targets unintended tissues or organs.

Immunocontraceptive Vaccine Design

General considerations for vaccine design for immunocontraception include the type of immune response desired, the nature of the antigen, and the method of delivery.

The panel considered two general approaches to immunocontraception:

1. Induce a one-time immune response to destroy targeted cells and induce permanent infertility.
2. Induce lifelong antibody production to interfere with GnRH function, block fertilization, or similar.

Each of these might require a different type of response; for example, inflammation would not be desirable when the target is in the brain, such as the neurons where GnRH is produced. Alternatively, a large inflammatory component might be required if the desired result is cell loss or tissue damage in the ovary.

Ways to activate T cells in addition to B cells were discussed. John Schiller mentioned the A□ vaccine that was tried in Alzheimer's disease. The antigen contained a sequence that was determined to induce an unwanted inflammatory response; by changing the sequence of the antigen, scientists were able to remove this T cell activating function while retaining B cell activation.

In general, small peptide hormones such as GnRH should allow direct antibody access, but as self proteins they may be tolerizing. John Schiller presented an approach to vaccine design incorporating repetitive antigens, a multivalent design in which at least 12 antigen repeats are spaced 50 – 100 Å apart. Such a design presents antigen in a way that resembles the native repetitive spacing on bacteria and viruses, and directly activates B cells providing a long-lived B-cell response. This method can be used to overcome tolerance to self antigens. Gregg Dean expressed concern regarding dangers associated with having a long-lived response to a continually produced antigen such as GnRH. John Schiller suggested that since GnRH is expressed at relatively low abundance, this might not be a concern.

Concerns regarding antigens specific to the sperm and egg include antibody access, and tolerance. In the female, the amount of mucosal antibodies is low, and even lower during ovulation. The presence of appropriate antibodies in seminal fluid was questioned, and to induce auto-immunity in the male against developing sperm would require breaking the blood-testis barrier.

To destroy reproductive tissue would require a T-cell response in addition to an antibody-mediated response. Since there are usually no T helper cells for self antigens, a non-self antigen such as flagellin would have to be included in the vaccine if a cell-mediated response was also desired. Adjuvants can be used to increase the strength of the immune response.

To induce immunity to a self antigen, one might also need to overcome the inhibitory effects of regulatory T cells (Treg cells, defined on page 6). Ways to do this include disrupting Treg suppressive activity with endogenous or exogenous IL-2, or depleting Treg with anti-CD25 antibodies.

There was concern expressed about potential side effects, including pain or discomfort, due to chronic inflammation in the gonads.

Vaccine Types and Formulation

In vaccinating against infectious diseases, vaccines usually take the form of a live attenuated pathogen, a killed pathogen, a portion of a pathogen, or a purified toxin of the pathogen. In each case, the pathogen is foreign and so an immune response is generated.

In immunocontraception, the proposed antigens are self antigens (the exception being sperm proteins in the female), and so vaccine design becomes more complicated.

Vaccine types to introduce antigen include:

1. DNA vaccines, in which naked plasmid DNA encoding the protein or peptide of interest is introduced into cells, where it directs the synthesis of the antigen. DNA can also be introduced in a viral, bacterial or bacteriophage carrier.
2. Protein vaccines, including purified proteins, protein conjugated to KLH or flagellin, or recombinant protein. The protein antigen can be injected along with adjuvant, or incorporated into virus-like particles (VLPs), liposomes, bacterial membrane vesicles, or the surface of a bacteriophage.

Virus vectors used for vaccines include canary pox, which expresses proteins well and cannot replicate in mammals, replication-deficient adenovirus, and alphavirus, which is currently used in anti-cancer vaccine research.

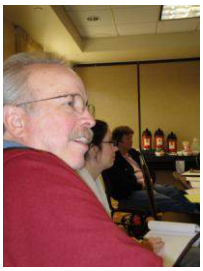
Bacterial vectors include Listeria monocytogenes and Salmonella spp. Roy Curtiss discussed a Salmonella vector his group has generated, which is engineered to remain in the cytosol, to express antigen only after colonization of lymphoid tissue has occurred, and to self-destruct to avoid shedding of live bacteria.

Adjuvants can be used to increase and sustain the immune response, but may not be needed if VLPs include endogenous DNA that can act as an adjuvant. Only three adjuvants are approved for use in humans – alum, squalene, and MPL – but additional adjuvants are allowed in animal vaccines.

To obtain 100% efficiency with a single dose usually requires a live virus or bacterial vaccine. In most cases, at least one booster is required several weeks after the initial inoculation. To get around this in an attempt to achieve an effective therapy that only requires treating the animal once, the panel considered the possibility of engineering an encapsulated dose that would be released at a future time. Gregg Dean recommended that biomaterials engineering groups be made aware of the interest in immunocontraception, since they would be the source of novel products for long-term or delayed release of antigen.

Practical Considerations

The regulatory aspects of vaccine development were discussed repeatedly, with emphasis on consideration of the feasibility of approval when beginning experimental design, including consideration of the responsible regulatory agency, which might be the FDA, EPA or USDA. For example, a treatment aimed at feral cats might fall under EPA regulation if the feral cats are considered animal pest species. Similarly, each component of a vaccine must be justified and approved, meaning it might be expeditious to pursue simple vaccines first, rather than trying to combine multiple antigens in an attempt to treat more than one sex or species.



Drs. Boyle and Coonrod, co-chairs of the think tank.

ACC&D Immunocon-traceptive Think Tank participants

Conclusions and Recommendations of the Panel

At the conclusion of the think tank, there were universal expressions of enthusiasm for the prospect for success in the immunocontraceptive approach to dog and cat sterilization. The panel agreed strongly on the following recommendations for a research agenda:

1. Support for the setting of practical and incremental research goals, including:
   - Targeting long-lasting contraception before permanent sterilization
   - Demonstration of effectiveness of a two-injection schedule before progressing to a one-shot treatment
   - Support for ideas that might work only in one species or one sex
2. Support for a collaborative effort among people from very different backgrounds, including engineering, reproductive biology, immunology, virology, etc., to attack a single problem, and the necessity to attract the best minds to the problem.
3. Support for further investigation into the application of GnRH vaccines to dogs and cats, applying modern vaccine design strategies, including phage display of repetitive epitopes to increase the strength of the immune response to vaccination, allowing for a longer-lasting effect. The panel noted the need for careful species-specific targeting, a requirement of any approach in which the target molecule is evolutionarily conserved.
4. Support for research into short-term cell-mediated responses to destroy reproductive tissue, inducing sterility. There was agreement that there may be much useful information in the past literature, where the goal was contraceptives as opposed to sterilants, and so treatments may have been abandoned that appeared to cause inflammation or permanent effects.
5. Funding for exploratory efforts involving proteomics or genomics to identify new markers specific for the cell types of interest and species of interest.
   - Interest in obtaining serum from dog or human cases of infertility and probing cat/dog tissues to find any reactive antibodies that might implicate autoimmune-related infertility
   - Research to characterize basic cat and dog immunology and reproductive biology, to better understand why previous vaccination strategies have not worked in these species
6. Support for exploratory proposals, since vaccinology is empirical and it is difficult to predict what will work.
7. Funding of parallel research into related technologies such as encapsulation/biomaterials or other methods of introducing a delayed release bolus for a second vaccine dose (booster) without needing to recapture the animal.
8. Identification and use of surrogate biomarkers of efficacy to speed immunocontraceptive research approaches.
9. Support for research in the target species, since there are known unique aspects of cat and dog biology, and immune reactions in these species may not be effectively modeled by experiments in other vertebrate species (e.g., mice).
10. Emphasis on a focus on safety, both of the treated animals, the environment, and the individuals who will administer the vaccine injection.
11. Consideration of FDA/EPA/USDA regulatory paths.
12. A recommendation that ACC&D consider other applications and partners – such as partnering with public health groups and organizations focused on controlling rodent pest populations.

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