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Vaccines for the control of reproduction--status in mammals, and aspects of comparative interest.

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The objective of producing vaccines which target elements of the reproductive system to control fertility has been pursued for many years. Of the many targets for such vaccines, several sperm-associated antigens have been proposed for antibody-mediated intervention before fertilization but the very abundance of antigen to be neutralized has been a barrier. Zona pellucida antigens associated with the surface of the oocyte have also been targeted and used successfully for control of 'wild' elephant populations but worries concerning immunopathologically-mediated tissue damage have been mooted. Vaccines using human chorionic gonadotropin (hCG) which is required for the implantation and maintenance of the fertilized egg, although of interest for the development of fertility control in human populations, has no relevance in the context of the present conference because external fertilization of fish eggs is independent. The pathways by which gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus promote release of luteinizing (LH) and follicle-stimulating hormone (FSH) which govern the physiological maturation and maintenance of the reproductive organs, provide many targets for immunological intervention. Most consistent success has been reported using GnRH-based vaccines which are immunosterilizing in a variety of mammalian species such as pigs, rodents and white-tailed deer. The fact that the structure of the decapeptide, GnRH, has been maintained over so many years of evolution and been conserved across so many animal species, encourages the view that a strategy for control of sexual maturation in fish based upon stimulation of GnRH antibodies may well prove to be a practical proposition, provided the formulation of an appropriate highly immunogenic vaccine can be achieved.

Concept and brief history of contraceptive and immunosterilising vaccines

The idea that vaccination against components of the reproductive system, such as gametes or hormones (Figure 1), could be used to interfere with sexual maturation or with fertility has been around for some time [1,2]. As far back as the 1920's and 1930's studies were being carried out even in the human species, involving injection of women with fresh semen or bovine sperm phospholipid in attempts to elicit immunological contraception. Research during the latter half of the 20th century has investigated the ability of vaccination to either arrest or impair sexual maturation, or to provide permanent or reversible contraception in sexually mature individuals. Several vaccines have now been tested for potential use in wild, feral and domesticated animal populations [3] and have also been developed for use in humans to provide an additional contraceptive choice for both men and women.

Much of the drive to develop such vaccines for use in humans has arisen due to the pressure of increasing world population, and the inadequacy of current contraception. Forty percent of couples of reproductive age do not practice any form of contraception. Even when contraception is used it is often not used as effectively as it could be. These facts result in fifty percent of the 1 million new pregnancies each day being unintended. Even in the USA, where access to contraception is comparatively straightforward, forty six percent of women have had at least one elective abortion by the end of their child-bearing years. Therefore, there is a clear need for the increased use of contraception and for a wider choice of contraceptive options.

With respect to animal populations, reduced fertility is often also desirable. The control of feral animal populations is a problem world-wide that is often difficult to achieve using conventional procedures such as shooting or poison bait. In the case of farmed and companion animals the

immunological control of sexual maturation or of fertility could be more humanely achieved by the use of vaccination rather than the often rather crude surgical procedures currently employed. Potential advantages of immunocontraception also include a lack of side effects seen with some other types of contraceptives, a long acting but reversible effect, ease of administration, cheapness, and for human use the fact that the vaccine can be confidential and not partner-dependent.

The immune response to contraceptive vaccines

Contraceptive vaccines need to stimulate the production of neutralizing antibody, and therefore require the recruitment of B-cells and helper T-cells in the absence of a response from potentially pathogenic cytotoxic T lymphocytes [2,4]. The antibody also needs to be present in the desired anatomical location. For immunosterilisation or immunocontraception based upon eliciting an immune response to hypothalamic or pituitary hormones then circulating antibodies will be sufficient, but at least for some gamete-based contraceptive vaccines the antibodies would need to be secreted into the reproductive tract. Optimal vaccination schedules for genital tract immunity in mammals are under intensive investigation, not least because of the importance of such studies to the prevention of sexually transmitted diseases in humans. Intranasal immunization appears to be a particularly effective route for the generation of antibody in the female reproductive tract.

Neutralising antibodies operate either by stearic hindrance (for example, blocking binding of a hormone to its receptor or of a spermatozoa to an oocyte) or by mediating the rapid clearance of the antigen via the formation of antibody-antigen complexes. Complement-mediated cytotoxicity, for example of sperm coated with complement-fixing antibody, may also be an objective. The vaccination of fish has now become a routine procedure, with vaccines available for protection against a variety of

infectious diseases including enteric redmouth (ERM), vibriosis and furunculosis. Although knowledge of immune responses in fish still lags some way behind our understanding of these systems in humans and rodents, their economic importance has provided much of the impetus for studying the fish immune system. It is known that fish possess both T and B lymphocytes with properties similar to their mammalian cousins. Thus, fish T-cells bear major histocompatibility complex (MHC)-restricted T-cell receptors (TCR). However, there are also some substantial differences. Whilst the B-cells of humans and rodents are capable of producing five different classes of antibody molecule (IgG, IgA, IgM, IgD and IgE), those of fish produce only a single IgM-like class of antibody which is produced in both tetrameric or monomeric forms. Peak titers of these antibodies occur five to six weeks following vaccination, and the antibodies can persist for up to one year, making immunosterilization or immunocontraception of fish an entirely feasible possibility. However, compared to most mammalian species, the development of immunological memory is a rather slow process, taking 3-6 months to be fully achieved. However, once established, this memory can last up to 8-12 months, enabling effective vaccination in this species.

Gamete vaccines

Both sperm and egg antigens have been used in a large number of experimental contraceptive vaccines for use in a variety of mammalian species. A major difference between mammals and fish is that antibodies to gametes in mammals could be targeted to act at any point prior to fertilization, whereas because most fish are oviparous the antibodies would need to act prior to the release of the eggs or sperm from the genital pore.

Spermatozoa vaccines

The rationale for using spermatozoa as a basis for contraceptive vaccines has to a large extent been based upon the observation that in humans naturally occurring anti-sperm antibodies are sometimes produced by the female partner, or sometimes the male partner, of infertile couples. Particularly noteworthy is the lack of side effects other than infertility seen in these individuals, suggesting that such vaccines would stand a good chance of being safe and acceptable. In mammals, antibodies in cervical mucus could cause agglutination of the spermatozoa and thereby prevent their access to the upper reproductive tract, whereas antibodies in the oviductal fluid could inhibit sperm-egg binding.

Although sperm-based vaccines are being developed for use in either female or males, there are a number of reasons why these might stand a better chance of working in females. Firstly, in female the antigen is 'non-self' and therefore conventional immunological tolerance does not have to be overcome. Related to this, as a safety issue, is that vaccination with a sperm antigen does not lead to an autoimmune response. Furthermore, the logistics are easier in the female because in the male there are 10^8-10^9 sperm that would need to be combated whereas in the upper reproductive tract of the female following coitus there are only tens or hundreds of sperm. Additionally, the immunology of male reproductive tract is poorly understood. In order to be effective, neutralizing antibodies would need to access the lumenal compartment of male reproductive tract. This adds some complexity to the system, although it might be possible to achieve this by inducing secretory IgA responses in the prostate.

The identification of sperm antigens as candidate molecules for inclusion in a spermatozoa-based vaccine has often centered around using antibodies from infertile couples either to screen cDNA

libraries made from testis or to probe high-resolution two-dimensional electrophoretic gels [5]. Using these and other approaches a large number of candidate antigens have been proposed [6] and homologous genes could be isolated from a fish testis cDNA library. Some encouraging results have been obtained using sperm vaccines, but generally a sufficiently high efficacy remains elusive. A vaccine utilising an immunodominant epitope from rabbit sperm protein 17 (SP17) reversibly reduced fertility in female mice from 72% in controls to 29% in the vaccinated animals [7]. Immunisation with another candidate antigen, fertilisation antigen-1 (FA-1), reduced fertility in female mice by up to 70% [8]. The use of a vaccine based upon a peptide from the sperm-coating seminal plasma protein inhibin abolished fertility in 75% of immunised male rats [9]. However, thus far the efficacy of sperm-based vaccines in nonhuman primates has proved to be rather poor. One solution to improving the potency of such vaccines might be to use DNA vectors encoding Th2 cytokines (e.g. IL-4, IL-5, IL-6) as B-cell adjuvants. The additional stimulus to the B-cell might induce sufficient levels of high-affinity antibody for effective bioneutralization of the spermatozoa.

Oocyte antigens

Most contraceptive vaccines based upon oocytes have employed the glycoprotein zona pellucida (ZP) antigens that are present on the outer surface of the egg. The three major molecules are ZPA (ZP2), ZPB (ZP1) and ZPC (ZP3). Such vaccines have proved to be effective but are quite often associated with T-cell mediated ovarian pathology, and with disturbances in the menstrual cycle, hormonal profiles and folliculogenesis. Therefore, attempts are being made to isolate relevant B-cell epitopes and use synthetic peptides representative of these epitopes [10]. However, a single B-cell epitope may not be sufficient for reliable contraceptive effect. Furthermore, most B-cell epitopes are discontinuous

are thus extremely unlikely to be adequately represented by a conventional synthetic linear peptide. However, not all studies with intact antigen have shown adverse side effects, and ZP-based vaccines are currently being developed for use in a number of species including cats, deer, horses, elephants, seals, wolves, bears, sheep, monkeys and rabbits [3,11,12].

One interesting success story using a zona pellucida vaccine involved the contraception of elephants in Kruger National Park in South Africa [13]. The elephant population is growing by over 500 each year, leading to overbrowsing of their habitat. In order to control their numbers the elephants were having to be culled, but this led to protests from tourists and from animal welfare groups. Therefore a trial of a vaccine based upon pig ZP was carried out. For the initial injection the elephants were immobilized using tranquillizer darts and at that time they were also fitted with radiocollars. This enabled them to be located by helicopters from which the booster injections were delivered by dart. When the boosters were given at 6 weeks and then 6 months, less than half the elephants given the vaccine became pregnant, whereas nearly all elephants given dart injections using a placebo became pregnant. If the boosters were given closer to the priming injection, at 2 and 4 weeks, the vaccine was even more effective. Furthermore, the contracepted elephants did not show behavioural changes that are seen in elephants given hormonal contraceptives [13].

One other particularly interesting example of a ZP-based vaccine are studies in which mice were immunized with a fusion protein containing the 200 amino acid N-terminal region of ZPA together with the sperm antigen Sp17 reduced fertility by 78% [14]. Although in this study the Sp17 acted primarily as a carrier, it was suggested that the use of other sperm antigens together with the N-terminal region of ZPA may produce an even more effective contraceptive vaccine.

Riboflavin carrier protein (RCP) vaccines

This vitamin carrier is present on ovulated oocytes and on spermatozoa. Vaccination of female rodents and primates with chicken RCP stimulates the production of antibodies which block pregnancy at around the time of implantation, or perhaps earlier during fertilization [15]. RCP is found also as a component of spermatozoa and therefore, might have additional potential as an antisperm contraceptive vaccine. Immunization of male rats and bonnet monkeys with RCP has been shown to decrease fertility, and several B-cell epitopes have been identified that could potentially be used in an RCP-based vaccine.

Hormone-based vaccines

The development of the gametes is under hormonal regulation and these hormones can also be inhibited immunologically (Fig. 1). Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus and promotes the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, thereby governing the physiological maturation and maintenance of the reproductive organs. These hormones therefore provide ideal targets for immunological intervention in animal species. In addition, human chorionic gonadotropin (hCG) has proved to be a very effective target in the human.

Gonadotropin-releasing hormone (GnRH)

A number of vaccines based upon mammalian GnRH are currently available, which can be used as either a means of preventing sexual maturation or as contraceptives [16-19]. Success has been

achieved using these vaccines in a variety of mammalian species such as pigs, cattle, deer, squirrels, and rats.

A 4-year study in male and female deer utilised a GnRH vaccine formulation containing keyhole limpet hemocyanin as a carrier together with complete Freund's adjuvant [18]. A booster was given one month later with the same vaccine except that the complete Freund's adjuvant was replaced by incomplete Freund's adjuvant which lacks the mycobacterial components of the complete adjuvant. Immunized male deer showed no interest in sexual activity, because the block in GnRH activity leads to decreased levels of testosterone. In immunized female deer there was an 88% reduction in the incidence of pregnancy. The vaccine was effective for up to two years without boosting.

A multimer of a modified GnRH sequence conjugated to the carrier ovalbumin and administered in Specol adjuvant efficiently immunocastrates young pigs, providing a humane alternative to surgical castration [19]. The fact that the structure of GnRH, which is a decapeptide, has been conserved during evolution encourages the view that a strategy for control of sexual maturation in fish based upon stimulation of GnRH antibodies may well also prove to be a practical proposition. Vaccines based upon Pisces GnRH have the potential to arrest or delay sexual maturation in salmon and other species of fish, with the delay in sexual maturation producing fish with improved gastronomic qualities. Success will, however, depend upon the formulation of the vaccine components in a way that will be highly immunogenic in these species. Thus, such a vaccine will need to stimulate the production of sufficient levels of high affinity antibodies in order to achieve effective bioneutralization.

Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) has the potential to be used as a male contraceptive vaccine. Successful studies have been carried out in both rats and monkeys [20]. A small clinical trial in men showed that production of FSH-specific antibodies could be achieved in all immunized individuals, although the vaacine in its current formulation for use in human subjects was not potent enough to cause a reduction in sperm count.

Human chorionic gonadotropin (hCG)

Human chorionic gonadotropin (hCG) is required for the implantation and maintenance of the fertilized egg in humans, but this hormone has no relevance to the external fertilization biology of the fish. Nevertheless, no discussion of contraceptive vaccines would be complete without mentioning this hormone as it forms the basis of the only contraceptive vaccine to have proven efficacy in humans [21].

This glycoprotein hormone consists of common α-chain also utilized by follicle-stimulating hormone, luteinising hormone and thyroid stimuating hormone, and a hormone-specific β-chain. The FSH, TSH and LH β-chains have extensive sequence homologies, respectively, 36%, 46% and 85% to the first 114 of the 145 amino acid residues of the hCG β-chain. The remaining C-terminal amino acids of the hCG β-chain are unique to hCG and referred to as the C-terminal peptide (CTP). Clinical trials utilizing hCG have employed three vaccines containing different versions of this pregnancy-associated hormone [1,2]. (1) A synthetic CTP corresponding to the unique carboxy-terminal 37 amino acids of the hCG β-chain, (2) The entire hCG β-chain and (3) the hCG β-chain combined with the ovine (sheep) α-chain to create a heterospecies dimer (HSD). The HSD version of the vaccine

remains the only contraceptive vaccine of any type to have successfully completed phase II efficacy trials in humans and convincingly establish the priciple of a vaccine approach to family planning [21].

Passive immunization

The vaccines described above represent agents that are used to provoke an immune response in the recipient, i.e. active immunization. An alternative approach is passive immunization in which preformed antibodies are administered to the animal. Passive administration of antibody has the advantage that the effect is immediate, rather than having to wait for the immune response to build up before a contraceptive effect is obtained as is the case following conventional immunization. Furthermore, there is no requirement to break immunological tolerance, and individual variations in immune responses and therefore in the level and duration of effectiveness are eliminated. However, passive immunization is likely to be more expensive and requires repeated administration. Some means of allowing continual release of the antibody would probably need to the employed in order to optimise this approach; one future strategy might be to express soluble antibody in commensal vaginal organisms. A humanised antibody capable of neutralising hCG has recently been produced for use in passive immunization. Passive immunisation has also been proposed for antibodies directed to spermatozoa antigens, such as for a hybridoma-derived monoclonal antibody against a sperm glycoform of CD52; the sperm agglutination antigen-1 (SAGA-1) [22].

Some issues relating to contraceptive and immunosterilising vaccines

The potential use of immunosterilising and contraceptive vaccines raises a number of issues. The main concerns are safety and efficacy. Most contraceptive vaccines depend upon the purposeful induction

of autoimmunity. For many of the vaccines described above this does not seem to have resulted in undesirable side effects. However, this is not always the case as the examples of ovarian pathology following immunization with zona pellucida antigens demonstrates. The issue of efficacy may be less stringent for target animal species than for use in human populations. Virtually all the vaccines that have been developed for contraceptive use in sexually mature individuals do not have a permanent effect. This is a desirable property. However, a man or woman using such vaccines for family planning purposes would need to be assured that the contraceptive vaccine will be effective during the period of time for which they do not wish to be fertile, and they would need to know how long the effect will last until they need to have a booster injection. The current generations of vaccines do not meet these criteria in that a significant minority of indivuals are poor responders to the vaccine and therefore not protected from pregnancy, and the period of effectiveness is not predictable. Thus, further development is required before routine use in human populations can even be contemplated. However, even an 80% reduction in fertility might be perfectly acceptable in some situations pertaining to animal species.

Conclusions

Based on progress so far, it would seem highly probable that, in at least some animal species, immunological sterilisation or contraception will become adopted as a humane addition to the methods used to control population levels. For captive animals, the use of GnRH has so far proved to be the most widely successful approach to controlling fertility. Further progress will, however, be required in the development of targeted methods of administration if this approach is to be extended to non-captive animals, such as rodent pests. The feasibility of using contraceptive vaccines in humans has been established but issues remain regarding overall efficacy and general acceptability.

Here, hCG has progressed furthest. As an alternative, the provocation of an effective anti-sperm response in females would be a highly desirable goal but success seems some way off. Combined vaccination against several antigens may ultimately prove to be the only way to ensure complete protection from pregnancy.

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Figure legend

Gonadotropin-releasing hormone (GnRH) produced by the hypothalamus stimulates the production of gonadotropin 1 (Gn1) and gonadotropin 2 (Gn2) from the pituitary. These gonadotropins (equivalent to mammalian follicle-stimulating hormone and luteinising hormone) act upon the gonads and thereby stimulate the production of the gametes. These hormones, and the gametes themselves, are amenable to immunologically-mediated intervention.