ADVENTS IN CONTRACEPTION : IMMUNOCONTRACEPTION

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ABSTRACT

Immunocontraception is one of youngest branch of immunology and represents the novel approach to the development of family planning methods. Investigations on Immunocontraception field endure in last hundred years due to revolutionary advancement was made with apparition of genetic, molecular biology and reproductive immunology. Such products would have many advantages over existing contraceptives in that they would not elicit metabolic disturbances, would provide long-acting (i.e. 6 to 12 months) protection from pregnancy and can be used by either men or women. Several lines of research and development currently in progress are aimed at the development of safe and effective immunocontraceptives based on reproductive hormones, components of the gametes (sperm and ova) and products of the early pre-implantation concepts. Antigenic basis of the first contraception vaccine was whole cells or tissue extracts, so that the most important antigen of the vaccines was not been precisely defined. There are several advancement of Immunocontraception relating classical approach in problems of contraceptives.

Recent developments in immunocontraceptions are the possibility of controlling fertility by antibodies inactivating key reproductive hormones has been amply demonstrated by active and passive immunization in primates. The further development, manufacture and distribution of immunocontraceptives will probably require the collaboration of public sector agencies, governments and industry in order to overcome the current paucity of effort being put into the development and provision of new, safe, effective and acceptable methods of family planning. The purpose of this review is to provide information on the current status of research and development of potential immunocontraceptives and to attempt to stimulate pharmaceutical companies to reassess their positions with regard to the development, manufacture and distribution of these products.

KEYWORDS: Immunology, Contraceptives, Antigen, Vaccines, Immunization,.

INTRODUCTION

Immunocontraception is non-hormonal form of contraception, based on the same principles as disease prevention through vaccination. An immunocontraceptives causes the production of antibodies against some essential element of the reproductive process, thus preventing pregnancy. The regulation of human and animal population numbers constitutes a difficult and largely unsolved contemporary problem. In the developed world, steroid contraceptives for humans are both widely used and efficacious. Elsewhere they are too costly. The development of less expensive methods is considered necessary [1].One such method is Immunocontraception, i.e. the vaccination against sperm, eggs, or reproductive hormones to prevent either fertilization or the production of gametes. Attempts to design human Immunocontraception have a long history [2]. The targets include sperm antigens, oocyte antigens, especially zona pellucida proteins (PZP), gonadotropin riboflavin carrier protein, gonadotropins and gonadotropin releasing hormones [3]. The most advanced method involves immunization against human chorionic gonadotropin, in reality a method of very early pregnancy termination [4]. It now seems likely that problems associated with autoimmune disease and variability of response will prevent any widespread use of Immunocontraception in humans in the foreseeable future[5] . Women's health advocates have objected to all forms of Immunocontraception because of perceived health risks and the potential for political abuse of the vaccine [6]. Human male immunocontraceptives have received much less attention and do not appear to be feasible in the near future [7].

Immunocontraceptives for wild animals have a different objective than those for humans. Their main aim is to check population growth rather than to contracept particular individuals. If some animals are irreversibly sterilized so much the better whereas such an effect in human medicine would be ethically most undesirable. Immunocontraceptives for animals are ostensibly humane and could potentially be used on the large scale required for wildlife population regulation. Research progress to date has been reviewed

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in Tyndale-Biscoe (1991, 1994), Barber & Fayrer-Hosken (2000), Barlow (2000), and Cooper & Herbert (2001). Three fundamental questions remain to be addressed: (1) Can sufficiently strong immune responses be provoked against the antigens (immunogens) of gametes or reproductive hormones to cause contraception in a proportion of animals large enough for effective population management? (2) How rapidly will variation in these responses lead to the evolution of failure to respond to the immunocontraceptives agent? (3) What will be the ecological consequences of the likely changes to the immunogenetic constitution of the population as a result of selection for non-responders? In particular, will the endemic pathogens of the species change? There is considerable information which allows us to answer at least in part the first two questions. The third is of fundamental importance but even a preliminary answer is not possible at present.

BASICS OF IMMUNOCONTRACEPTION

The neonatal vertebrate's immune system develops recognition of "self" proteins, carbohydrates, and hormones. This self recognition is essential, since the production of antibodies against pathogenic bacteria and viruses is necessary for survival. However, the formation of antibodies against "self" can be an abnormal destructive process, e.g., diseases like multiple sclerosis and arthritis.

Anti-fertility vaccines are directed against "self" reproductive antigens (hormones or proteins) to which the recipient normally is immunologically tolerant. These antigens are made "non-self" or "foreign" by coupling them to a protein that is recognized as foreign to the animal. As the animal' immune system examines the conjugated self-foreign protein, antibodies are produced to its own reproductive proteins and hormones. This induced immune response against "self" is the key to Immunocontraception. The infertility lasts as long as there are sufficient antibodies to interfere with the biological activity of the targeted hormone or reproductive protein, usually 1-2 years.

(a) Target species

Population control of native and exotic mammals is generally justified by environmental degradation, competition with and predation on native wildlife, conflicts with humans over food production, potential spread of pathogenic infectious diseases and the possibility of population crashes of over-abundant fauna or of

wildlife populations near urban areas. Although still in its infancy, Immunocontraception is regarded as being more humane than the traditional methods of wildlife population control, such as shooting, trapping, poisoning, or pathogenic agents and its use has strong support from influential animal welfare agencies worldwide [8].

(b) Immune responses to self-antigens

Responses to self-antigens are unusual and mainly weak. This constitutes a major barrier to the development of an immunocontraceptives. The reason for this could be either genetic or environmental. In either case it indicates that a fraction of the population will continue to breed despite the administration of the contraceptive. In most cases, there is likely to be at least in part genetic causes underlying lack of response. If so, the genes for lack of response will be selected for and in a comparatively small number of generations most of the population will be non-responsive. This implies that the immunocontraceptives can be useful for only a short period of time. The need for multiple injections and the dependence upon adjuvant to achieve the necessary level of response renders the whole approach impractical at present. The most commonly used adjuvant, Freund's adjuvant, also induces a range of undesirable side effects and its use is being challenged on animal welfare grounds [9]. There is at present no feasible or acceptable method of promoting responses to self-antigens sufficient to cause Immunocontraception.

Jackson et al.(2001) attempted to overcome the problem of lack of immune response to self-antigens in the absence of adjuvant by inserting the cytokine interleukin-4 into mouse pox virus with the intention of increasing the humeral response. The virus was then inserted into the mice with the unwelcome outcome that the mice all died very quickly. This work caused alarm because of the possibility that this technology could lead to a method for simple conversion of relatively innocuous viruses into lethal ones, which could be used in biological warfare [10].

Another possible problem with virus-vectored Immunocontraception is the potential for the horizontal transfer of the immunocontraceptives gene into viruses affecting other species . While it may be possible to create genetically modified organisms without adverse effects on the target animals, the effects they might have on related species they come in contact with make any use of this approach questionable.

(c) Variation in response and genetic change

Variation in to bio control agents is a widespread phenomenon. This variation has frequently led to evolution of a degree of resistance so that the agents is no longer useful. The evolution of resistance to the insecticide has been reviewed by McKenzie (1996).He draws a distinction between bio control agents with responses within the phenotypic range and those with responses outside the phenotypic range of the target species. An agent who initially kills all members of the target species is acting outside the normal phenotypical range while one which kills only a fraction of the population is acting within the normal phenotypical range. He points out that when the resistance appears in the former case it is frequently monogenic, while in the latter case a number of different genetic regions are involved, i.e. it is probably multi-factorial. The basic genetic parameter to be estimated in either case is heritability, i.e. the extent to which genetic variation is controlled by genetic as opposed to environmental factors. The relative fertility of the immunocontracepted animals is >10% in 27 out of the 32 data sets. A proportion of non-responders are characteristic of most species. Only three species (Tammar wallaby, Fallow deer and Norway rat) out of 14 had no non-responders . Following McKenzie's (1996)argument, this implies that non-response is likely to be multi-factorial in genetic terms. There are no data which will allow estimates of the heritability of non-response to Immunocontraception in any of the species. We are unable to predict the rate at which this characteristic will increase in any one of these populations under the selective influence of Immunocontraception. However, some idea of the likely change per generation given the initial frequency of non-responders can be found using Falconer's (1965) model for threshold characters. Reproduction is a good example of a threshold character; it is an all-or-none attribute which can be affected by a variety of underlying genetic and environmental factors. If heritabilities are high, rapid selection occurs. This is shown by the high percentage of non-responders that occur within one generation.

HERITABLE VARIATION AND CONTRACEPTION RESISTANCE

Traits that vary are not always heritable. For any quantitative trait heritability is defined as the extent to which the phenotypic variance in a trait (V_P) is a consequence of genetically caused, as distinct from environmentally caused, variation (V_G as distinct from V_E). In the broadest sense it

can be thought of as the proportion of the variance of a trait attributable to genetic causes. Note that in'contracept population' fertility variation will not be all-or-none. Individuals will vary in the level of induced Immunocontraception. Heritability is normally expressed as V_G/V_P, where $V_P = V_G + V_E$. The genetic variation component is normally further subdivided into three components, that due to additive effects (V_A), dominance effects (V_D) and genetic interaction (epistatic) effects (V_I). In predicting the response to selection, the additive effects are considered particularly important because they determine the similarity between parents and offspring; in other words the extent to which variation for a trait in one generation is passed to the next generation. Interaction effects can also have an impact on resemblance across generations. Because of the importance of V_A, the extent to which a trait is genetically determined is often expressed as V_A/V_P, defined as the narrow-sense heritability. An important point is that if a high proportion of the population phenotypic variation is caused by environmental factors (V_E is large) then even intense selection will have little consequence in changing the phenotype of future generations. While levels of contraception resistance may vary markedly among individuals this does not mean heritability is high.

Immunocontraception research has largely ignored the heritability of fertility effects. However, there are heritability data on one component of the immune response, the antibody response, and there are some studies of associations between antibody response and resultant fertility. It is important to distinguish between heritabilities of these two measures because only effects on fertility are selected; non-response of antibodies will not be selected in the absence of associated fertility effects. Also, while antibody response to an immunocontraceptives treatment may be a primary mechanism that reduces fertility, it is unlikely to be the only mechanism since other processes such as cell-mediated immunity may be involved.

Heritabilities of antibody levels have been estimated from selection experiments. For example, selection of chickens for high and low antibody response to two different bacterial vaccines was carried out for seven generations. All high response lines exhibited significant increases in antibody production to both vaccines when compared to the low lines. Unfortunately the omission of a control line means that we cannot be confident the increase was other than a response to general rearing conditions. Assuming

this was not the case heritabilities for antibody response were low (ranging from -0.23-0.61). In a similar chicken experiment, selection over a period of 14 generations was the likely cause of high and low antibody titres attained and heritabilities ranged between 0.02 and 0.20.In mice selected for high and low response to antigens for 15 generations, immune responses were 60 times higher in high lines compared to low lines. Heritability was estimated at 0.18. Similar antibody heritabilities were estimated for pure-bred pigs immunized with two different vaccines. Here low heritabilities of 0.18 ± 0.09 and 0.15 ± 0.07 were recorded for immune response to a modified-live pseudo rabies vaccine and an inactivated bacterial bacterin, respectively, both measured 56 days after inoculation (however, it was 0.52 ± 0.15 for the bacterin after 119 days). Overall the data indicate that the level of an antibody response is heritable and will respond to selection, but heritabilities are not necessarily high relative to other traits.

Immunization followed by a booster can elicit an immune response that results in reduced or zero fertility, provided antibody titres remain high. However, in some cases a high immune response did not ensure against contraception resistance, there was no apparent correlation between antibody titre and fertility or associations between these traits were ambiguous because appropriate controls were either absent or inadequate. These data indicate that high antibody titres do not ensure contraceptive efficacy. However such associations might be higher in future efforts to relate fertility to immune response levels if the efficacious epitopes are identified and quantified.

While the heritability for antibody response levels are low to intermediate, the heritability of fertility effects are likely to be even lower, since antibody levels are only one component of fertility effects and underlying components of traits have higher heritabilities than the traits themselves. This might be particularly the case under field conditions where environmental variance is likely to be higher and contribute to each subcomponent of the fertility trait. While most studies of heritable variation in non-response have taken place in defined laboratory situations, natural conditions will be more stressful and/or more variable, reducing the heritability of contraception resistance. Also, levels of heritable variation expressed in one environment are not necessarily the same as those in another environment. Considering the above, in all likelihood contraception resistance will have a low

heritability and therefore respond only slowly to selection.

Note that small sample size is an area for concern in the estimation of heritabilities for immunological responses. Heritabilities have usually been estimated using a few individuals and these estimates have little statistical power. They may be unrepresentative of a population, especially given the large amount of genetic polymorphism associated with immunological responses to contraception. Estimates for out bred animals tend to be particularly variable (for example, natural variability in pregnancy rates of foxes after mating was between 35 and 90%).Note also that heritabilities are not constant but change as selection proceeds because they depend on underlying gene frequencies that change under selection. Predictions of selection responses based on heritabilities therefore only apply for a few generations. Finally, note that heritabilities do not necessarily provide information on the extent to which a trait will shift under selection; the extent of change is also influenced by the mean of a trait.

(a) Genetic basis of contraception resistance and selection intensities

Heritable variation alone does not ensure the evolution of a particular phenotype. The type and level of genetic variation and the intensity of selection are also important. If selection intensities are relatively high, a response to selection may not be successful and the population will go extinct even when there is some genetic variance in the population. There are several cases in the pesticide resistance literature where resistance has not evolved or has not persisted despite the presence of genes with small effects on resistance variability and strong selection pressures. Strong selection may kill all individuals unless they carry major resistance genes (although see Groeters and Tabashnik). A similar situation is thought to be relevant for the evolution of resistance to other toxicants, such as heavy metals. High selection intensities can favour the spread of genes having large effects on a trait, and genes that are often initially rare, particularly if the genes show some dominance. However, local populations may not be large enough to harbour such individuals and evolution does not occur. The frequencies of genetic variants, the nature of the way the genes affect the phenotype and selection intensities are all important for predicting the occurrence and speed of evolutionary change.

The genetic and molecular basis of Immunocontraception resistance has yet to be explored. However genetic variation in some

components of the immune response, especially antibody response, has been investigated. Early research suggested that the genetic basis for antibody response is under the control of a dominant Mendelian locus. Later data suggested both a dominant autosomal effect of a major gene and some polygenic influences. The MHC complex, containing an array of tightly linked genes that are highly polymorphic, appears to be a strong candidate for a major autosomal dominant gene that affects antibody response. However non-MHC linked genes, such as those involved with Class I antigen processing or genes that influence antibody isotope usage, may have substantial effects. Therefore the genetic control of antibody non-response might be realistically modelled as two or more independent (unlinked) polymorphic genes, one with a major effect on fitness, the MHC cluster, and several with minor effects.

For contraception resistance on the other hand, where associations between antibody response and contraception resistance may not be strong, other genes again are likely to be involved. For example, genes that effect variation in cell-mediated immune responsiveness or expression of cytokines (reviewed in Reiner and Locksley). A further level of complexity is introduced if the antigen is delivered using a genetically modified vector organism, in which case the target species may evolve resistance to the vector itself. The evolutionary genetics of host resistance to a virulent horizontally transmitted pathogen is well-researched and modelled and may help our understanding of contraception resistance evolution in these situations. While single genes can be important in host-pathogen systems, this complexity makes the prediction of contraception resistance even more difficult. It may be appropriate to model contraception resistance using a small number of major genes and a large number of minor genes. Polygenic models that rely on an infinite number of loci each with small effect, or simple single gene models, are likely to be unrealistic.

(b) Proportion of the population that is contraceptive resistant

To predict the outcome of selection for contraception resistance it will be important to know both the initial frequency of resistant individuals and the ongoing impact of immigration from non-immunized sources, from 'refuges', that can dilute the frequency of resistant individuals. Such inward gene flow could prevent resistance evolution. The proportion of a population that retains any level of fertility after contraception

treatment is likely to be highly variable. For a given immunocontraceptives in a particular population it is this proportion that determines the initial selection response. If the initial frequency is very low resistance will build up slowly and populations may become extinct because they do not reach effective demographic population size. Data useful for predictive purposes need to be obtained from out bred animals in the field, as laboratory-reared or inbred populations will have less genetic variability affecting contraception resistance. Also, captive populations are likely to show high fertility levels, having been selected for breeding in captivity and buffered against natural stresses that decrease fertility.

Because average fertility levels of non-immunized individuals are below that of the most fertile individual, one must assume that the functional or effective level of contraception resistance (remaining fertility) is higher than the published data indicate. For example, using data from fox, the effective contraception-resistance fertility after immunization would be close to 53% (36%, of control fertility at 68%), and for the feral mares the effective level of fertility would be between 8 and 51% (between 4.5 and 28.6% of about 55%). We suggest that the effective contraception resistance levels be used in any evolutionary modelling exercises. However, these levels might be overestimates since breeding failure in controls may be related to other factors (for example, lack of opportunity or poor conditions compared to treated individuals).

To predict the results of selection for contraception resistance, information on immigration from refuge populations is needed. Gene flow often limits adaptation, as has been recognized for a number of traits and organisms. An influx of susceptible individuals into a population previously exposed to controlling treatment will dilute the frequency of resistance genes. The dilution effect is likely to be particularly significant in the case of polygenic resistance. When individuals with polygenic resistance mate to susceptible individuals, the gene combination needed for resistance will be lost in the ensuing generations because there is only a low probability of recovering offspring with polygenic resistance. This dilution effect has a much lower impact in the case of major genes. The dilution effect will depend on the species, population, and environment being considered. For example interpopulation movement level is likely to be very different between the red kangaroo and the brush tail possumtion Clearly in captive populations gene

flow will have no influence on the response rates to selection for contraception resistance.

CROSS GENERATION EFFECTS, RESISTANCE TRADEOFFS AND COST OF CONTRACEPTION

Cross-generation effects can have a negative impact on selection responses. Cross-generation effects arise when environments experienced by the parent influence the phenotype/performance of the offspring, or when genes carried by the parent (usually the mother) influence offspring phenotype (even when the offspring has not inherited a causal gene). Often this results in a fitness cost in the F1 generation that can influence selection responses. They can even lead to phenotypic changes in the direction opposite to that being selected.

The potential for an immunocontraceptives vaccine to have cross-generation effects is suggested by one study on rats. Both males and females were subject to vaccination against luteinizing hormone receptor (LHR), and mated with untreated individuals. For both treated sexes the number of progeny per coupling was reduced compared to control groups but was more apparent in the couplings of a treated male. A cross-generation fertility effect was evident for the F1 progeny from treated females where, in the absence of vaccine, the F1 progeny had reduced fertility. Such effects have the potential to reduce the selective intensity for contraception resistance and alter the rate of evolution of contraception resistance.

(a) Genetic trade-offs

Much of life history theory is concerned with genetic tradeoffs and how they limit evolutionary change. There is a lot of evidence that alleles favoured under one set of environmental conditions can be selected against under a different set of conditions thus leading to tradeoffs between environments. Moreover, genetic change in one trait often has costs in terms of a different trait even when the environment does not change. There are good examples of tradeoffs associated with resistance to chemicals. For instance, while both warfarin resistance in rodents and pesticide resistance in insects lead to increased survival when these poisons are present in the environment, resistance is often selected against when the chemical is absent resulting in the loss of resistance in populations that are no longer exposed to the toxin.

Subsequent fitness improvement that counteracts these deleterious effects may occur in the longer term however, when treatment persists. This is particularly well studied in the case of sheep blowflies where resistance to the pesticide diazinon is associated with a fitness cost. In this example there is no fitness cost detectable in the genetic background of a contemporary field population where the pesticide has been present for many years. In this population genes have been selected that modify the deleterious effects of the major resistance gene. If a major gene for contraception resistance was associated with fitness costs and reached high frequency in a treated pest population, modifier gene selection could nullify the cost, helping to maintain the gene in the population and thwart control efforts.

Little research has been conducted into defining or clarifying potential tradeoffs for Immunocontraception resistance. This issue is pertinent given that a low level of responsiveness to some antigens could potentially influence responsiveness to others. These individuals may be more susceptible to particular categories of infectious or parasitic agents that retard selection for contraception resistance, particularly if the target population experiences intermittent applications of the vaccine. With intermittent selection, any change in contraception resistance gene frequencies could theoretically be reversed during periods when the immunocontraceptives agent is absent.

Tradeoffs will only limit adaptation if they have a genetic basis. To establish this in mammals individuals should be followed over two or more generations and sample sizes need to be large. While for morphological traits there may be a good correspondence between simple phenotypic tradeoffs associations and genetic correlations, this is not the case for other traits making it difficult to test for genetic tradeoffs involving Immunocontraception resistance.

(b) Loss of genetic variability and immunological fitness

A possible outcome of long-term selection for contraception resistance is a general loss of genetic variability. In the laboratory, strong selection for a single trait can reduce levels of genetic variability in that trait as favoured alleles go to fixation. While in theory this would lead to a reduction in trait variance it often does not. Intense selection can also lead to a general decrease in genetic variation if the number of breeding individuals in a population is small. However there is also evidence that sharp reductions in population size can increase levels of

trait variability due to the 'unveiling' of no additive genetic variance. A reduction in population size as a consequence of Immunocontraception may therefore not invariably decrease phenotypic variability.

Another possible outcome of long-term selection for contraception resistance is a loss of immunocompetence. As the fertility response to an antigen may be largely controlled by the MHC locus, individuals resistant to contraception could show reduced diversity in their immunological response. At this stage a few laboratory investigations have reported that selection for high antibody responders is associated with altered immunological fitness against other antigens/pathogens, but this is not a general finding. If hypersensitivity to a specific infectious agent was recognized tradeoffs for a contraception resistance gene, a synergistic treatment program (treatment with both vaccine and infectious agent) could become part of a management control strategy, similar to what has been implemented for integrated insect pest management schemes.

Under field conditions, the outcome of contraception-resistance selection on levels of genetic variation in target and other traits is difficult to predict. Effects are likely to be species-specific and dependent on the duration and intensity of selection. To determine the impact of such selection on levels of genetic variation and overall immuno-competence it will be necessary to undertake both field and laboratory-based experiments looking at longer term effects. Of course loss of genetic variability and immunological fitness will be less of a problem for invasive or exotic pest populations targeted for elimination.

Also note that within the lifetime of immunized individuals deleterious direct effects of immunization may occur that lead to behavioural changes or low 'quality of life', effects that are over-and-above those that reduce fertility. These changes may or may not be an undesirable outcome of the Immunocontraception; however, they can effect social dynamics within the population and impact on selection for contraception resistance.

THE COMPONENT OF THE POPULATION TARGETED FOR CONTRACEPTION

The proportion of a population immunized will impact directly on the rate of increase of contraception resistance in a population; since the selection intensity will be reduced if not all breeding individuals are targeted. If the species to be controlled is contained within restricted habitats, it may be possible to inoculate the entire population. However in many cases this will be impossible. Several modelling and laboratory investigations have suggested that, to obtain the necessary reduction in population size, approximately 50–75% of individuals need to be targeted. If only a small proportion of the breeding population is targeted effectively, selection intensity for increased levels of contraception resistance will be low.

The sex that is targeted can influence potential selection intensity. In most case studies female-specific contraceptive agents have been tested although males have also been investigated. When the immunogen targets the reproductive response of both sexes, there will be fewer progeny from contraception-resistant individuals. However selection for contraception resistance will be greater as all progeny will contain contraception-resistant genes from both parents, as opposed to only the treated sex.

The social and reproductive dynamics of the targeted population will influence selection intensities for resistance. For example, under a harem system where a dominant male mates many females, control programs may target the dominant male in the group. Alternatively, when reproduction occurs in distinct times of the year, it may be more effective to only target populations at these times, perhaps when climatic or nutritional stress is maximum. These approaches could more effectively reduce population size without increasing selection intensity for contraception resistance. Knowledge of a species' social and mating structure can help the design of a strategy to minimize resistance evolution.

(a) Optimal contraception requires multiple and booster immunizations

Multiple applications of an immunogen are required to elicit the maximum level of infertility response. This is generally the case for a range of laboratory and out bred species. Thus to achieve high levels of infertility individuals need to receive multiple applications, spread out over time. In addition, immunocontraceptives effects have a finite duration and booster vaccinations are required to maintain a high level of infertility. This is particularly relevant for species with a long generation time and/or multiple reproductive seasons – additional factors that would impinge on the timeframe for the evolution of Immunocontraception resistance. While the goal for much of the research is for a long-lasting single shot vaccination this does not yet appear to have

been achieved. It is therefore likely that in natural populations it may be difficult, even impossible, to implement complete control by a single treatment or repeated regular delivery of the immunogen to many or most individuals. In large or complex populations, a number of strong short-term selective episodes at intermittent times would be interspersed with periods of reduced selection for resistance. If there are genetic tradeoffs associated with contraception-resistance there may even be negative selection at these times, decreasing the chance of resistance evolving.

(b) Multiple vaccines in rotation or mixtures

A multiple-vaccine approach has been advocated as a strategy to improve contraception and population control; it might also minimize the chance of resistance evolving. This strategy has been successfully used to reduce the rate of insecticide resistance evolution in field situations. By rotating pesticides, the number of applications of a particular chemical is reduced. This effectively reduces the selection pressure for resistance to each pesticide and also allows any fitness costs of resistance alleles to decrease resistance incidence between applications. Moreover, any influx of susceptible individuals into a target population in the intervening period can further reduce the incidence of resistance. The other strategy involves mixing pesticides. This may delay the evolution of resistance more effectively than pesticide rotation strategies—however, if resistance occurs it is more likely to elicit a single cross-resistance response. Pesticides with a contrasting mode of action are the best choice, whether rotating or mixing, since a common mechanism of resistance is more likely for related pesticides. The experimental and theoretical aspects of a multiple-treatment approach with respect to pesticides have been discussed by Tabashnik.

Exposure to multiple antigenic determinants may therefore be effective; particularly where the mode of action of the immuno-contraceptives varies and the same genes are unlikely to confer cross-resistance. While in some systems a response to a particular antigen carries a similar high response to other antigens, this is not always the case, as has been demonstrated in mice. A range of antigenic determinants within a vaccine might elicit diverse contraceptive-effective responses within individuals, and thus decrease the fertility level of the average individual, and the frequency of contraception-resistant individuals. In fact the growing understanding of the complexity of fertility controlling processes, and growing number of candidate target molecules, are leading to suggestions that multiple antigen approaches will be most effective in future immunocontraceptives efforts. Multiple genetic bases for contraception resistance will be more likely under this approach and resistant individuals would be rarer. As well as improving control a multiple antigen approach would retard the evolution of contraception resistance.

WHAT IS THE CURRENT STATUS OF DEVELOPMENT OF PROTOTYPE IMMUNOCONTRACEPTIVES?

Immunocontraceptives vaccine can control reproduction at various stages. They can interrupt the reproductive activity of both sexes by

(a) Interfering with the biological activity of hormone

(b) Blocking sperm penetration of an ovulated egg, or

(c) Preventing implantation and development of a fertilized egg.

Possible points of intervention

Hypothalamus-		GnRH
Pituitary	-	FSH and LH
Gonads	-	progesterone, estrogens and testosterone
Gametes	-	ovum and sperm
Pre-embryo	-	structural and endocrine components

(a) GnRH immunocontraceptives:- Various veterinary trials to control feral animal populations and for immunological castration. Gonadotropin releasing hormone (GnRH) is produced in the brain by the hypothalamus and controls release of the pituitary reproductive hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones in turn control the hormonal functions of the gonads (ovaries and testes). Antibodies to the hypothalamic hormone will reduce the circulating level of biologically-active GnRH, thereby reducing the release of subsequent reproductive hormones. The reduction or absence of these hormones leads to atrophy of the gonads, resulting in infertility in both sexes. Both avian and mammalian forms of GnRH have been identified.

Clinical trial conducted in postpartum women to prolong an ovulation.

Clinical trial conducted in men with prostatic cancer.

Clinical trial in healthy men

(b) FSH immunocontraceptives

Phase I clinical trial conducted in normal men to assess immunogenicity and to assess effect on spermatogenesis

Prototype preparation found to be only weakly immunogenic, some reduction in sperm numbers and motility but no significant effect on semen parameters

(c) Steroid immunocontraceptives

Several studies carried out in laboratory animals but no known clinical trials conducted to date

(d) Gamete immunocontraceptives

The zona pellucida (ZP) is a cellular glycoprotein surrounding the egg or oocyte. It is located on the outer surface of the egg between the oocyte and the granulose cells. Antibodies to this glycoprotein layer result in infertility by 1 or both of these actions: (a) blocking sperm from binding to the ZP layer, and (b) interfering with oocyte maturation. For a sperm to fertilize the egg, it must first bind to a receptor on the ZP. An enzyme in the sperm breaks down the ZP and allows the sperm passage into the ovum. Antibodies to the ZP also prevent fertilization by interfering with oocyte-granulose cell communication, resulting in the death of the developing oocyte (Dunbar and Schwoebel 1988).

Since protein in the sperms' head normally binds to the ZP receptor on the oocyte, antibodies to these sperm proteins can be produced, by vaccination in the female that are available to bind to sperm in the oviduct. This prevents sperm from binding to the ZP receptor. Sperm protein Immunocontraception is being investigated for contraception of the red fox and the rabbit in Australia (Morell 1993, Tyndale-Biscoe 1991). A ZP protein has not been identified in avian species, nor has the cross-reactivity of PZP been tested in avian species. Chorionic gonadotropin (CG) hormone, which is produced by the implanting embryo in some species, induces the corpus luteum to continue production of progesterone which is required for the maintenance of pregnancy. Antibodies to CG reduce blood levels of this hormone and thereby prevent implantation of the fertilized egg. Some cell surface antigens are unique, tissue-specific, immunogenic and accessible to antibodies:

- Zona pellucida antigens: used in animal control
- Sperm antigens: naturally-occurring antibodies lead to infertility

No known clinical trials conducted to date.

(e) Pre-embryo immunocontraceptives

The riboflavin requirement of the developing embryo is satisfied by active transport of this water- soluble vitamin across the placenta. This transport is provided by a gestational-specific carrier protein called riboflavin carrier protein (RCP). RCP plays a pivotal role in embryo development in avian and mammalian species. Antibodies formed against RCP interfere with placental transfer of riboflavin, thereby preventing development of the early embryo. This technology probably would result in the least change in social behaviour of the target species of any of the proposed vaccines.

(f) hCG immunocontraceptives

Several types and formulations of hCG-based immunocontraceptives have been studied extensively in preclinical studies and clinical trials sponsored by:

National Institute of Immunology, Delhi, India

Population Council, New York, USA

World Health Organization, Geneva, Switzerland

METHODS OF ADMINISTERING VACCINES

Subcutaneous or intramuscular (I.M.) injection is the traditional forms of vaccine delivery. In order to accomplish I.M. injections in free-roaming animals, the vaccine must be delivered by a dart or a "bio-bullet" (Kirkpatrick et al. 1990, Turner and Kirkpatrick 1991, Garrot et al. 1992, Turner et al. 1991, 1992). While these methods may be effective in certain confined locations, they are impractical when dealing with mobile wildlife populations in large open areas.

Except for the oral polio vaccine introduced by Dr. Sabin in the 1950s, oral vaccination has received little attention for humans because it requires larger quantities of vaccine and is less predictable than subcutaneous or I.M. routes. In mammals, oral immunization takes place in the pharyngeal immune follicles (e.g., the tonsils) and in the small intestine. There are thousands of immune follicles throughout the small intestine, with a higher concentration in the distal portion in most species. Vaccines, being protein in nature, are digested rapidly in the stomach when given

orally; hence, immunization must occur either in the pharyngeal area or the vaccine needs a protective capsule to survive passage through the stomach then be released in the small intestine (McGhee et al. 1992).

The safest way to deliver the antigen orally is to protect it until it is taken up by the PP and delivered to macrophages. A combination of 2 approaches could lead to effective antigen uptake and potentiation of mucosal immune response: (a) enteric coating of the antigen resulting in delivery vehicles that prevent degradation in the stomach but allow absorption in the intestine, and (b) designing the vaccine to have enhanced attraction to the immune follicles in the small intestine.

Recent understanding of the mechanisms by which pathogenic viruses and bacteria colonize and infect the intestinal tract has provided new insights for developing successful and safe attenuated live or killed oral vaccines. For example, bacteria must survive the stomach's acid and proteolytic enzymes to successfully infect the small intestine. After surviving intact through the stomach, it must have adhesive properties which allow it to adhere to and colonize the intestinal wall, resulting in an infection. Bacteria without adhesive properties will be carried out of the gut with the waste material.

Liposomes are spherical, artificial biological membranes made up of phospholipids and cholesterol that can be used to protect oral vaccines from digestive tract degradation. Since the liposome membrane contains lipids, which are stable in the gastrointestinal tract, an antigen placed inside during liposome synthesis is protected from gastrointestinal degradation. Cholesterol in the membrane adds stability and makes it attractive to macrophages in the PP where the liposome is taken up rapidly because of the membrane's lipophilic nature. This characteristic of the membrane causes the liposome to simulate a microbial cell when presented to the immune system. The liposome acts as an antigen micro carrier capable of targeting the antigen directly to the PP.

However, before a liposome can be taken up by the macrophages, it must bind to the mucosal surface of the intestine; otherwise it will be swept out with the waste material. This mucosal adhesive property increases the mucosal uptake efficiency, thus requiring a smaller oral vaccine dose. The most commonly used liposome adhesive is a nontoxic form of the bacterial lectin, cholera toxin (CT), a member of a family of enterotoxins produced by several strains of enter pathogenic

bacteria (Holmgren et al. 1992). Lectins have multiple binding sites and can bind to receptors on the liposome as well as to intestinal receptors.

Recent advancements in molecular biology and immunology have provided us with new tools such as "live vectors" as delivery vehicles. The most prominent use of this technology in wildlife management is the use of the live vaccinia virus to deliver rabies vaccine orally to raccoons (Procyon lotor) and foxes (Vulpes vulpes). The attenuated vaccinia virus, a member of the pox viruses, was used as a vaccine against smallpox in humans for over 20 years. Using recombinant genetic engineering, the gene responsible for encoding of the rabies virus glycoprotein was inserted into the vaccinia virus by scientists at the Wistar Institute. This recombinant pox virus, when given orally, was able to vaccinate the target animal against rabies. The tonsil lymphoid tissue is thought to initiate the immune response in these target animals (USDA-APHIS 1991).

Live viral vectors potentially can be used to deliver a contraceptive vaccine. This delivery system is currently being tested in Australia (Tyndale-Biscoe 1991).

ADVANTAGES OF IMMUNOCONTRACEPTIVES

- lack of endocrine or metabolic side-effects;
- do not require insertion of an implant or device;
- provide long term but not permanent protection;
- do not require storage or disposal by the user;
- use is independent of coitus;
- permit confidentiality of use;
- low annual cost to users and services.

DISADVANTAGES OF IMMUNOCONTRACEPTIVES

- Delay between administration and attainment of effective immunity;
- Need for periodic injections;
- Individual variations in immune responses and, therefore, in level and duration of effectiveness;
- Cannot be 'turned off 'on demand;
- Not a barrier to sexually-transmitted infections/HIV;

- Alleged abuse potential and other socio-political issues.

THE PATHWAY OF DEVELOPMENT

These include:

(1) Fundamental discovery and characterization of appropriate immunogens derived from reproductive hormones and/or from the sperm, egg, egg investments, conceptus, or accessory reproductive organs

(2) Development of methods for producing the immunogens to high standards of purity through

   (a) Genetic engineering of genes encoding specific immunogens

   (b) Peptide syntheses

   (c) Isolation of the antigen from natural sources

(3) Production and purification of immunogens under good laboratory practices (GLP)

(4) Formulation of immunogens doses

(5) Small animal and primate testing of immunogen formulations for immunogenicity, safety, and efficacy

(6) Evaluation of mechanisms of immunogen action

(7) Human trials for immunogenicity, safety, and efficacy, using formulations produced under good manufacturing practices (GMP)

(8) Development of diagnostics to monitor infertility status in recipients of effective immunogens

RESULT AND DISCUSSION

Immunocontraceptives will probably overcome the demerits of current contraceptives with respect to safe, effective and acceptable methods of family planning.It seem likely that the use of Immunocontraception will result in selection pressure for contraception resistance. The development of contraception resistance will vary among target species and may only occur over such a long time frame that changes in control approaches or community priorities remove the need for concern. However until levels of heritable variation in contraception resistance are assessed in each particular situation, and unless other pertinent factors that are likely to impinge on resistance evolution are evaluated, it is not clear if or how rapidly contraception resistance will develop in a population. The other pertinent factors include:

1. the genetic basis of the resistance

2. the frequency of resistant individuals, both initially and on-going as influenced by immigration from non-contracept refuges

3. fitness costs associated with the resistance phenotype

4. cross-generation effects, and

5. the efficiency of delivery of the vaccine

If enough information is available on the contraception response and population biology of the target species it should be possible to implement strategies for reducing the rate of evolution of contraception resistance.

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