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Theoretical Article

Immunocontraception for population control: Will resistance evolve?

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Summary The prospect for successful biocontrol using immunocontraception is threatened if there is adaptation to the vaccine through natural selection of individuals that are genetically resistant to the contraceptive agent. To assess this possibility we examined the literature and found that little relevant data are available for any species on the appropriate trait, fertility variation among immunized individuals, or about appropriate population and genetic parameters influencing the likelihood of a selection response. Some data are available on variation in antibody response to immunocontraceptives, but the relationship between antibody response and fertility levels is poorly documented. The antibody response data indicate low heritability for this trait suggesting that fertility levels of contraceptive-resistant individuals will also have a low heritability. Slow evolution of contraception resistance might therefore be anticipated. The absence of information about relevant parameters makes the construction of quantitative models premature. We discuss factors in particular need of investigation if predictions about resistance evolution are to be made. These include: 1. the genetic basis of fertility retention, 2. the proportion of the population resistant to the contraceptive agent and how this is affected by gene flow from refuge populations, 3. the genetically-based fitness tradeoffs of resistant individuals that often accompany selection, 4. cross-generation effects that can thwart the effects of selection, and 5. the efficiency of delivery of the contraceptive agent. An understanding of the above for particular species, and the development of appropriate divergently acting multiple vaccines that can be used in temporal rotation or in mixtures, should facilitate the development of management options to minimize resistance evolution.

Key words: immunocontraception, population control, resistance evolution.

Introduction

Environmental degradation and economic losses due to pest populations of native and exotic mammals are widespread problems[1,2] and immunocontraception has been heralded as a potential solution to these problems.[3–5] Immunocontraceptive vaccines are designed to target the reproductive system of pest mammals by promoting antibody production against various proteins necessary for reproduction.[6] The potential end result is a publicly acceptable and humane control over wildlife numbers. Nevertheless, the application of this method to wildlife populations is still in its infancy and several concerns have been raised regarding detrimental consequences of immunocontraception.[7] One of the most controversial issues involves selection for individuals who fail to mount a significant contraceptive response to the immunogen and hence remain fertile in the presence of the vaccine. It is possible that the progeny of these individuals will inherit this resistance in which case alleles of genes responsible will increase in frequency. Thereby biocontrol via immunocontraception will become increasingly ineffective over time due to strong selective pressures in favour of non-response to the immunocontraceptive.[8]

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There are many examples of rapid evolutionary changes in populations faced with an environmental shift, such as the evolution of pesticide resistance in insects, resistance to anticoagulants in rodents, and resistance to heavy metals in plants and invertebrates.[9–11] Adaptation to environmental shifts, however, can also be unsuccessful. Many fossil studies suggest that species move away from stressful conditions rather than adapt to them.[12–14] There are also many documented examples of population extinction following stressful conditions.[15,16] Even in the case of pesticides and fungicides, resistance is not necessarily a foregone conclusion as there are agrichemicals that have been used for many years without resistance problems being encountered. Thus it is not clear that resistance adaptation to immunocontraceptives is inevitable.

To address the likelihood of resistance evolving a number of factors and population processes need to be considered. While extensive research has been conducted on developing immunocontraceptive vaccines, far less research has been directed towards assessing population variability in fertility response to vaccines, and towards identifying factors that might influence resistance evolution. Many such factors are intrinsic properties of the species or population, such as the degree to which contraception resistance is heritable. Other factors however, might be manipulated in ways that minimize resistance evolving, as has been done for pesticide resistance.[17]

Here we elaborate on the factors and processes that need to be understood to assess the likelihood of the evolution of

contraception resistance. We draw upon examples from other types of traits and from non-mammalian species and make suggestions for research directions to address concerns. Once relevant factors and processes are understood, strategies could be put in place that reduce the likelihood of this occurring.

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 a 'contracepted 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.[18] 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 immunocontraceptive 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.[19] 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.[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.[21] Heritability was estimated at 0.18. Similar antibody heritabilities were estimated for pure-bred pigs immunized with two different vaccines.[22] Here low heritabilities of 0.18 ± 0.09 and 0.15 ± 0.07 were recorded for immune response to a modified-live pseudorabies 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.[23–25]

Immunization followed by a booster can elicit an immune response that results in reduced or zero fertility, provided antibody titres remain high.[26–28] However, in some cases a high immune response did not ensure against contraception resistance,[29–31] there was no apparent correlation between antibody titre and fertility[32] or associations between these traits were ambiguous because appropriate controls were either absent or inadequate.[29] 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.[24] 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.[33] 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.[34] 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.[30,35] Estimates for outbred animals tend to be particularly variable (for example, natural variability in pregnancy rates of foxes after mating was between 35 and 90%).[36] 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.[25,37]

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.[38] 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.[39] Strong selection may kill all individuals unless they carry major resistance genes (although see Groeters and Tabashnik).[40] A similar situation is thought to be relevant for the evolution of resistance to other toxicants, such as heavy metals.[41] 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 frequency 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.[42] Later data suggested both a dominant autosomal effect of a major gene and some polygenic influences.[43,44] 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.[45,46] 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.[47] 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,[48] or expression of cytokines (reviewed in Reiner and Locksley).[49] 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[50,51] 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. More information is needed if models relevant to natural populations are to be developed.

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.[52,53] For a given immunocontraceptive 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 outbred 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,[52] the effective contraception-resistance fertility after immunization would be close to 53% (36%, of control fertility at 68%), and for the feral mares[53] 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.[54–56] 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[57] and the brushtail possum.[58] Clearly in captive populations gene flow will have no influence on the response rates to selection for contraception resistance.[55]

Cross generation effects, resistance tradeoffs and costs of contraception

Cross-generation effects

Cross-generation effects can have a negative impact on selection responses.[59] 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[60] that can influence selection responses. They can even lead to phenotypic changes in the direction opposite to that being selected.[61]

The potential for an immunocontraceptive vaccine to have cross-generation effects is suggested by one study on rats.[62] 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.

Genetic trade-offs

Much of life history theory is concerned with genetic tradeoffs and how they limit evolutionary change.[25] 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[15,25] 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.[60,63,64]

Subsequent fitness improvement that counteracts these deleterious effects may occur in the longer term however, when treatment persists.[9,10] This is particularly well studied in the case of sheep blowflies where resistance to the pesticide diazinon is associated with a fitness cost.[65] 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 immunocontraceptive 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.[18] While for morphological traits there may be a good correspondence between simple phenotypic tradeoff associations and genetic correlations, this is not the case for other traits[25] making it difficult to test for genetic tradeoffs involving immunocontraception resistance.

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.[18] 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 nonadditive genetic variance.[66] 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.[7,8] 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.[21,67] If hypersensitivity to a specific infectious agent was a recognized tradeoff 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.[68]

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.[7] 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.[69–71] 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[27,31,72] although males have also been investigated.[62,73] 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.

Optimal contraception requires multiple and booster immunizations

Multiple applications of an immunogen are required to elicit the maximum level of infertility response.[28] This is generally the case for a range of laboratory and outbred species. Thus to achieve high levels of infertility individuals need to receive multiple applications, spread out over time. In addition, immunocontraceptive effects have a finite duration and booster vaccinations are required to maintain a high level of infertility.[74] 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.

Multiple vaccines in rotation or mixtures

A multiple-vaccine approach has been advocated as a strategy to improve contraception and population control;[30] 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.[17,75,76] 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,[77] however, if resistance occurs it is more likely to elicit a single cross-resistance response.[78] 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 has been discussed by Tabashnik.[79]

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.[80] 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 immunocontraceptive efforts.[5,81,82] 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.

Conclusion

It seems 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-contracepted 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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