Developmental and reproductive toxicity testing of vaccines

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ABSTRACT

The majority of new preventative and therapeutic vaccines are now assessed for developmental toxicity according to guidelines issued by the FDA in 2006. Despite the absence of confirmed effects in humans, vaccines are frequently suspected of having adverse side-effects on the development of children. Such suspicions are perhaps unavoidable considering the extremely widespread use of vaccines. The preclinical developmental toxicology studies are designed to assess possible influences of each component of the vaccine formulation—and the induced antibodies—on the development of the conceptus, neonate and suckling organism. Immune modulation by a vaccine or an adjuvant could, for instance, affect the outcome of pregnancy by interfering with the natural shift in immune balance of the mother during gestation. Maternal immunoglobulins are transferred from the mother to the offspring in order to confer passive immunity during early life. This maternal antibody transport is prenatal in humans and monkeys, but tends to be delayed until after birth in other species. Therefore, a suitable model species needs to be chosen for preclinical studies in order to ensure exposure of the foetus to the induced maternal antibodies following vaccination. Rabbits are the best laboratory model for prenatal immunoglobulin transfer, but rodents are more practical for the necessary postnatal investigations. Non-human primates are the only appropriate models for the testing of vaccines that are not immunogenic in lower species. It is advisable to test new adjuvants separately according to the ICH S5(R2) guidelines. Preclinical paediatric investigations are not currently required for vaccines, even though most vaccines are given to children. Other areas of regulatory concern include developmental immunotoxicity and effects on the preimplantation embryo. Because of the limitations of the available animal models for developmental toxicity testing, pharmacovigilance is essential.

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1. Background

The first technical guidance on the preclinical testing of preventative and therapeutic vaccines for developmental toxicity was issued by the CBER Division of Vaccines and Related Products Applications of the FDA in 2006 (FDA, 2006a). Before this, developmental and reproductive toxicity studies were rarely performed for vaccines. This approach was not entirely justified, since vaccination programmes at that time often included pregnant women (Gruber, 2003). Influenza vaccines, for example, were already being recommended for use during pregnancy by public health policy makers in the absence of specific regulatory approval for use of the product during pregnancy (Centers for Disease Control and Prevention, 1999). This practice was brought to the forefront of public attention by the recent worldwide H1N1 vaccination programmes (Centers for Disease Control and Prevention, 2009).

The first draft of the FDA guidance document was issued for comments in 2000 (FDA, 2000). A Workshop was organised jointly by the Society of Toxicology and the FDA in 2002 to discuss advances and regulatory considerations in the non-clinical safety evaluation of preventive vaccines. During the course of this meeting the possible risks of adverse effects of vaccines on human development were reviewed and recommendations were made concerning the need to develop new animal models and methods (unpublished). Many of these recommendations were subsequently incorporated into the final guidance document (FDA, 2006a). The European guidelines for the nonclinical testing of vaccines issued in 1997 (EMEA, 1997) state that embryo-foetal and/or perinatal studies may be necessary for vaccines that will be given to women of child bearing age or during pregnancy, but give no guidance on study designs. In the absence of guidance from the regulatory authorities of other regions, the FDA developmental toxicity study designs have become de facto the international standard for the testing of new vaccines.

2. Possible risks of vaccines to development

To date, there is no documented causal evidence of developmental or reproductive toxic effects in humans following the use of an approved vaccine. Live vaccines are contraindicated during pregnancy because of the risk of infection of the conceptus. The inadvertent use of smallpox vaccine during pregnancy, for instance, carries a risk of foetal vaccinia, but does not appear to result in birth defects

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or preterm delivery (Ryan et al., 2008). Despite the lack of causal evidence, vaccines are frequently suspected by the general population of having adverse side-effects on the development of children; the most recent point in case being the suspected implication of a swine flu vaccine in childhood narcolepsy (Daily Telegraph, 2010), even though the epidemiology data are reassuring (Schaefer, Fritzsche, Karbaum, Meister, & Weber-Schoendorfer, 2010). Such suspicions are unavoidable considering the extremely widespread use of vaccines in children, a small proportion of whom will inevitably fall ill following vaccination. Some longstanding concerns, such as the possible implication of vaccine products in the increasing incidence of childhood allergy, are more plausible than others (Offit & Hackett, 2003). Even in these cases, however, the minor unproven risk of increased sensitivity to allergy is far outweighed by the health benefits of vaccines in conferring protection against life-threatening disease (Forster, this issue).

The extremely long-lasting pharmacological efficacy of vaccines—i.e. lifelong or long-term immunity—presents unique considerations that are not applicable to other classes of medicinal agent. Following vaccination, the induced antibodies and/or sensitised T-cells or memory cells typically persist for decades. Also, unlike most other pharmaceutical agents, an administered vaccine often exerts its pharmacological action at the site of administration (e.g. an intramuscular injection site) and does not necessarily need to enter the general circulation in order to be effective. Thus, conventional pharmacokinetic measures of dose and exposure are not applicable. Antibody titres are measured for vaccines as a substitute for the pharmacokinetic and pharmacodynamic parameters evaluated for conventional drugs. Each component of a vaccine formulation—antigen, adjuvant, excipients, vehicle, etc.—needs to be evaluated for potential adverse effects on development. This is usually accomplished by evaluating the final vaccine formulation, though in most circumstances it is advisable to also test an adjuvant separately (see below).

An additional complication is the need to assess the possibility that the induced antibodies may have the potential to cross-react with endogenous tissues due to molecular mimicry between the infectious organism and the human host. Developmental toxicity could arise when the affected host cells play an active role in a developmental process. Furthermore, the endogenous antigenic molecules may only be present during restricted periods of development, leading to phase-specific effects. For this reason, it is necessary to expose the model organism throughout its entire development (i.e. up to maturity) in developmental toxicity testing to be sure of covering all possible periods of vulnerability. An example of molecular mimicry occurs between polysaccharide Group B Neisseria meningitides (GBM) vaccines and mammalian neural cell adhesion glycoproteins (NCAMs) (Verdier, Barrow, & Bruge, 2003). Polysialiated NCAMS play an important role in the remodelling of various tissues at specific times during development. Once development of the tissue is complete, the NCAMS are deactivated by removal of the sialic acid polymer. Mouse antibodies raised against GBM polysaccharide have been shown to cross react with activated human NCAMS, but no adverse effects on development have been demonstrated in-vivo. Exposure of the embryo to maternal antibodies is prevented by the placental barrier (see below), so adverse effects of cross-reacting antibodies during organogenesis are unlikely. However, NCAMS control some critical aspects of development that occur later in development, e.g. brain maturation or secondary sexual development of the testes, when maternal and/or endogenous antibodies are present.

Most vaccines are designed to provoke a humoral (adaptive) immune response. Adjuvants are designed to enhance this response, either through immune-modulating effects or by improving the presentation of the antigen. Immune stimulation by either the vaccine antigen or a co-administered adjuvant may alter the natural balance between the innate and adaptive arms of the immune system. The resting balance of the maternal immune system shows a natural shift during pregnancy in order to reduce the risk of rejection of the embryo and to accommodate the normal immune interactions between the maternal tissues and the developing conceptus. During pregnancy, cellular immune activity, modulated by TH1 cells, is depressed while humoral immune activity, modulated by TH2 cells, is increased (Thellin & Heinen, 2003). Specific immune stimulation by a vaccine antigen or non-specific stimulation by an adjuvant could conceivably interfere with the shifted balance during pregnancy and thus adversely influence the outcome of gestation. Cytokines of macrophage origin, for example, have been shown to cause pregnancy loss and abortion (Raghupathy, 1997).

On the other hand, non-specific immune stimulation of the mother with adjuvants or cytokines, such as GM CSF or IFNγ, has been shown to have a protective effect in laboratory animals against the dysmorphogenic action of many known teratogens (Holladay et al., 2002). The mode of action of this protective effect remains to be elucidated.

It is reasonable to assume, therefore, that vaccines—or adjuvants—may have the capacity to affect the outcome of gestation, owing to their inherent pharmacological activity on the immune system. The end result of these influences may be beneficial or detrimental for the developing conceptus or child.

3. Animal models

Passive immunity conferred from the mother serves to protect the newborn from infectious disease during early life, until the infant's own immune system becomes fully functional. The conferred immunity involves the transfer of maternal immunoglobulins (mainly IgGs) from the mother to the offspring, which takes place at different times relative to birth in various mammalian species. This transfer is essentially prenatal in the human. Old World primate species also show prenatal maternal antibody transfer, as do lagomorphs (rabbits and guinea pigs). Rodents, however, have very limited prenatal maternal immunoglobulin (Ig) transfer, while the transfer is entirely postnatal in most other species (e.g. dogs, cats and pigs) (Pentsuk & van der Laan, 2009).

In early gestation, the placental barrier prevents exposure of the developing embryo to maternal immunoglobulins and the embryo does not have the capacity to produce its own antibodies. Therefore, the induced antibodies following vaccination are unlikely to cause dysmorphogenesis via a direct interaction with the embryonic tissues during organogenesis. The human chorioallantoic placenta develops active transport mechanisms for IgG—via the FcRn receptor—starting from about mid gestation, after which an interaction with the developing foetal tissues becomes a possibility (although the period of vulnerability to most teratogenic agents is already over). Non-human primate species show a similar placental function as humans. In rodents and lagomorphs, the FcRn receptors reside in the inverted yolk sac (or vitalline) placenta. Rabbits have the unique characteristic of transporting small, but significant quantities of IgM to the foetus in addition to IgG (Baintner, 2007). In humans, other primates and rabbits, the foetal IgG levels generally reach, or exceed, the maternal titres by the time of birth. Prenatal IgG titres are much lower in rodents, but reach maternal titres within a few days after birth (Halliday, 1955).

On the basis of the above considerations, primate species are the most appropriate models to study of the effects of vaccines on intrauterine development. There is increasing ethical pressure, however, to avoid the use of monkeys in pharmaceutical safety testing whenever possible. Rabbits or rodents are generally preferred for this purpose and are also much more practical than primates for reproductive toxicity testing (Barrow, 2009). Rats and mice are the most practical species when postnatal examinations are required, although more expertise in the area of postnatal studies in rabbits has been acquired over recent years (see below). Rabbits have a higher foetal exposure
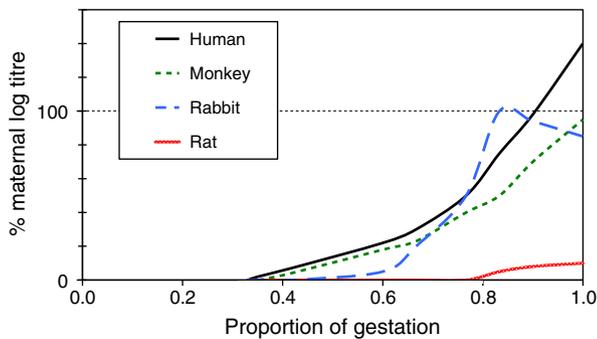
Fig. 1. Schematic representation of foetal immunoglobulin G levels during gestation in humans, non-human primates, rats and rabbits (redrawn from Pentsuk & van der Laan, 2009).

to induced antibodies (Fig. 1). Clearly, the chosen species must mount an immune response to the vaccine. For vaccines that are not antigenic in lower species, non-human primates may be the only appropriate models (see below), otherwise their use is not justified. In other cases, the choice of the rat, mouse or rabbit will probably depend on the degree of foetal exposure to the induced maternal antibodies that can be attained under experimental conditions.

4. Regulatory guidelines

The European Agency for the Evaluation of Medicinal Products issued a guidance document in 1997 on the preclinical testing of vaccines (EMEA, 1997) which recommended developmental toxicity testing for vaccines intended for use in women of child-bearing age. No guidance was given on study designs, however.

In 2006, the CBER division of the FDA issued a guidance document on "Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications", which gives specific recommendations on study designs and experimental methods (FDA, 2006a). This document is generally used today as the sole guideline for the design of non-clinical developmental studies for vaccines.

The ICH S5 guidelines (ICH, 2005) are now applied worldwide for the reproductive toxicity testing of new pharmaceuticals for reproductive and developmental toxicity (Barrow, 2009). These ICH study designs, however, are not applicable for the testing of vaccines. A typical ICH embryo-foetal development study involves the daily dosing of pregnant rodents or rabbits from the day of embryonic implantation through to the end of organogenesis, followed by a

caesarean examination of the dams (Wise et al., 2009). Continuous exposure of the pregnant dam to the test item is considered necessary in order to cover all of the possible widows of vulnerability to drug-induced dysmorphogenesis during organogenesis. Such frequent administration of vaccines would result in a massive overdose with respect to the intended clinical use of the vaccine, and may also induce desensitisation to the administered antigen. Also, in order to test any possible influences of the induced antibodies on the course and outcome of pregnancy, it is necessary to vaccinate the animals before mating to allow sufficient time for the initiation of antibody production and ensure maximum exposure during pregnancy.

In the FDA study design, all possible adverse influences of the vaccine preparation on development are assessed in a single experiment. The animals are vaccinated once or twice before mating at intervals designed to result in peak maternal antibody titres during early pregnancy. Another dose of vaccine is then given after mating at around the time of embryonic implantation, to ensure exposure to the various components of the vaccine formulation during organogenesis. Finally, a booster vaccination towards the end of gestation ensures sustained maternal titres during lactation. It is generally accepted that a single species is sufficient for these investigations (as opposed to two species for the teratogenicity testing of pharmaceutical agents under to the ICH guidelines). A single dose level is normally tested in vaccine studies, which is preferably equivalent to a single human dose without scaling for body weight. The maximum administrable dose is often limited by the volume of vaccine formulation that can be given to the animal by the intended human clinical route. For vaccines intended for intramuscular administration, a total volume of 1 mL can be comfortably injected in the rabbit, 0.2 mL in the rat and 0.1 mL in the mouse, with half of the volume injected into each thigh. As for all nonclinical safety studies, a control group is given a placebo formulation. Each treatment group comprises two subgroups: half of the females are submitted to a caesarean examination at the end of gestation and half are retained for postnatal examinations. The recommended (but not required) group size is 20 pregnant females with viable young in each subgroup. Note that this group size is higher than that generally required for ICH studies with conventional drugs (20 litters per group versus 16) (Wise et al., 2009); the reason for this difference is not clear. Maternal and foetal blood samples are taken for the determination of vaccine-induced antibodies. In the case of multivalent vaccines, the antibodies to all valencies are normally assayed.

The caesarean subgroup is used for foetal examinations and routine teratology investigations (Fig. 2). Rats are submitted to caesarean
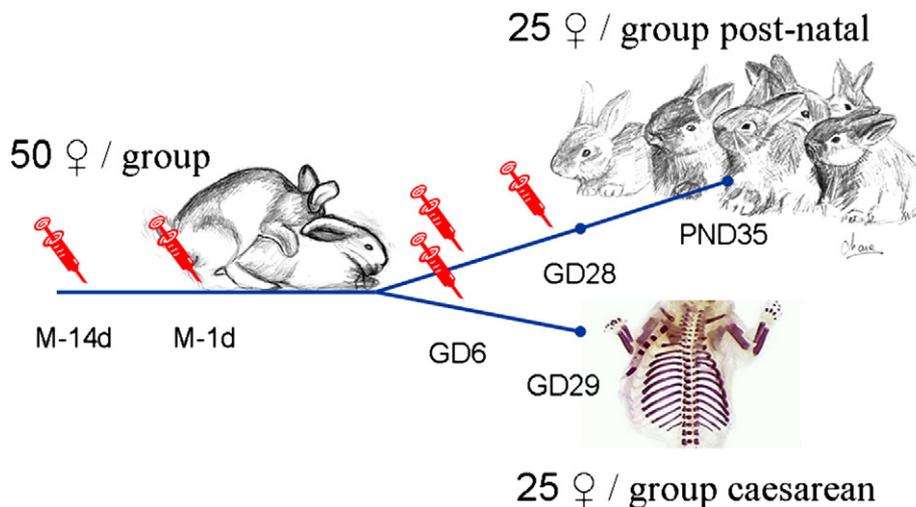

Fig. 2. Experimental design of a developmental toxicity study in the rabbit for a new vaccine. M = day of mating. GD = gestation day. PND = post natal day.

on gestation day (GD) 20, mice on GD18 and rabbits on GD29. The females in the other subgroup are allowed to litter and the development of the pups is monitored until weaning. The delivered pups are counted for each dam after completion of parturition. Any dead pups are removed from the nest and necropsied. The surviving pups are examined daily. Individual pup weights are recorded at least twice-weekly, starting from postnatal day (PND) 1 in rodents or PND4 in rabbits. Physical development of the pups is assessed by monitoring developmental milestones, such as the days of incisor eruption, fur growth and eye opening. Various reflexes of the pups are also tested, e.g. surface righting, auditory reflex and pupil response. The dams and surviving pups are submitted to a necropsy examination at weaning (at three weeks of age for rodents, five weeks for rabbits). The study may be extended to evaluate the postweaning development of the offspring if equivocal effects are seen during the previous phases.

It should be emphasised that the developmental toxicity study design described above is intended as an apical screen to detect any adverse effects on development, whatever the causative agent (antigen, adjuvant, induced antibodies, etc.) and whatever the mechanism. The study will hopefully detect all adverse effects on the course and outcome of gestation and on postnatal development irrespective of the mechanism e.g. direct toxicity of a component of the vaccine formulation, the induction of an immune response, or immune imbalance of the mother. In the case of identified toxicity, however, further studies will most likely be necessary to identify the causative agent and elucidate the mechanism. This is a good reason to test adjuvants separately (see below).

5. Species selection

Species selection is based upon the degree of foetal exposure to the induced antibodies that can be achieved. Rodent species are preferred if feasible in view of cost, ease of handling and acquired historical reference data. Even though the degree of placental transfer of antibodies is limited in mice and rats, an optimised maternal immune response (e.g. using a higher dose) may be sufficient to induce foetal antibody titres in these species that are equivalent to the anticipated foetal titres in the human. The use of the rabbit requires more technical expertise for the postnatal examinations, although initial difficulties related to maternal cannibalism following the handling of the kits have now been largely overcome with recent improvements in housing conditions and animal handling techniques. The guinea pig shows a much greater degree of cross-placental antibody transport than in the human (Pentsuk & van der Laan, 2009), but there is little or no experience with this species for regulatory developmental toxicity testing.

A preliminary study may be performed to compare the pre- and postnatal antibody transfer in rats, mice and rabbits following administration of the vaccine. Such a study typically includes groups of 12 dams of each species, half of which are used for foetal antibody determinations at the end of gestation and half for pup antibody determinations during the first week postpartum. On the basis of four such preliminary studies, the mouse was found to have suitable foetal exposure for two vaccines and the other two vaccines had to be tested in the rabbit due to either poor maternal immunogenicity or insufficient foetal antibody titres (Verdier et al., 2003). Recently, the rabbit has tended to become the species of choice, even when rodents have acceptable prenatal antibody transfer, since the larger size of the rabbit allows the administration of one human dose of the vaccine, which is not always feasible in rodents (see above).

6. Adjuvants

All components of the vaccine formulation, including adjuvants, solvents, stabilisers, preservatives and contaminants, have a potential to adversely affect development. It is important, therefore, to obtain developmental toxicity data for each component. For commonly used substances, this data will already be available in the literature or from studies previously performed by the manufacturer.

Even though the adjuvant component of a new vaccine will be tested in the developmental toxicity study as part of the vaccine formulation, it is nonetheless preferable to perform separate developmental toxicity studies, particularly for an adjuvant intended for use with several vaccines. Such studies should be performed according to the ICH S5(R2) guidelines (ICH, 2005). This will allow the adjuvant to be tested without the limitations imposed by the vaccine formulation (i.e. in a rodent and non-rodent species, using multiple dose levels with repeated administration throughout all critical phases of development). An embryo-foetal study should ideally be performed in the rat and rabbit, though one species alone may be considered sufficient, provided that adjuvanted vaccine formulations will tested in a second species. A fertility study, or a combined embryo-foetal and fertility study (Barrow, 2009), in a rodent species is also advisable. This study design evaluates fertility effects in both males and females. A pre- and postnatal study is usually considered superfluous, given the low likelihood of transmission of the adjuvant to the infant via the maternal milk.

7. Non-human primate studies

Developmental toxicity studies in primate species are best avoided if possible for ethical, economic and practical reasons. Primate studies are, nonetheless, unavoidable for any biotechnology vaccines that are not immunogenic in non-primate species.

Due to the expense and poor availability of mature non-human primates, relatively few animals can be used in each study. In addition, each pregnant monkey typically only has a single foetus (compared with an average of nine or 10 in the rabbit), so the number of offspring available for examination following exposure to a vaccine is severely limited. The statistical power of developmental toxicity studies in non-human primates is consequently severely limited. The studies are also of much longer duration and are more labour-intensive than the equivalent studies in rodents or rabbits.

Inspiration concerning a suitable study design for vaccines can be gained from an "enhanced pre- and postnatal" protocol (Stewart, 2009) proposed for the testing of monoclonal antibodies (Mabs) in the recently issued draft addendum to the ICH S6 guideline (ICH, 2009). In a similar vaccine study design (Fig. 3), female cynomolgus monkeys could be vaccinated at intervals throughout gestation and lactation, starting on GD20 (when gestation can be confirmed by ultrasound scan). All of the females would be allowed to give birth and the development of the offspring monitored for one to 12 months. The lack of foetal examinations following caesarean section is not expected to impact on the capacity of the study to detect
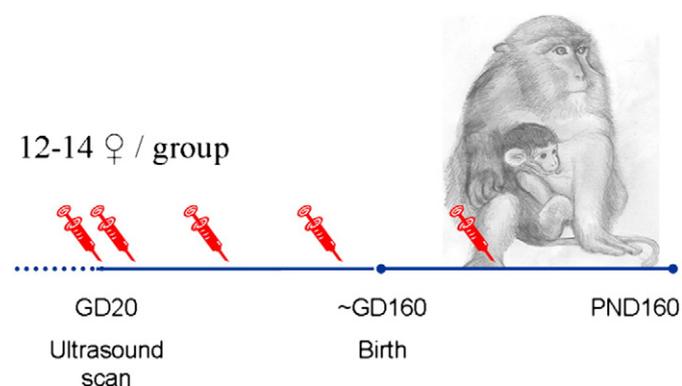

Fig. 3. Experimental design of a developmental toxicity study in the cynomolgus monkey for a new vaccine. GD = gestation day. PND = post natal day.

dysmorphogenesis of the offspring, since in-utero X-ray examinations are performed, the delivered babies are given a morphological examination at birth and any aborted or stillborn foetuses are recovered for examination. Vaccination before mating in monkey studies is impractical because of unpredictable menstrual cycling, uncertain copulatory performance and low fertility rates (Chellman et al., 2009). Luckily, there is no need to vaccinate before mating in view of the much longer gestation period of monkeys (about 100 days) compared with rodents and rabbits. Vaccination from GD20 would leave adequate time for maternal antibody titres to peak before the onset of placental Ig transfer during mid-gestation. Depending on the antigenicity of the vaccine, a vaccination scheme with two closely-spaced vaccinations at the start of gestation followed by booster vaccinations could be devised, with the aim of maintaining high maternal titres through to the end of the study. Blood samples would be taken from the mother and baby at suitable intervals for antibody assays.

8. Juvenile toxicity

A paediatric investigation is now mandatory for new drug applications in North America (FDA, 2006b) and for marketing authorisation applications in Europe (EMEA, 2008). There are currently no such requirements for vaccines, even though most vaccines are given to children. Children have been shown to be more sensitive than adults to many types of pharmaceutical-induced toxicity (Barrow, 2007). Nonclinical juvenile animal studies have two distinct objectives: 1) to detect any increased vulnerability of the immature animal to the systemic toxicity of the drug compared with the adult and 2) to assess adverse influences growth and development. The drugs of most concern are those that exert their therapeutic action on the organs that undergo prolonged postnatal development, or that have known secondary effects on those organs. Vaccines clearly fall into this class of concern for paediatric toxicity, in view of their numerous potential interactions with the immune system during its prolonged period of postnatal development (see below). It is likely that the attention of the authorities will shortly be turned towards the validation of relevant juvenile models for the nonclinical testing of vaccines.

Juvenile toxicity examinations on conventional drugs generally require a specific stand-alone protocol in an appropriate species. Such studies could be avoided for vaccines by integrating a juvenile investigation into the routine repeat dose (RDS) studies (Forster, this issue). This would involve using juvenile animals for the general toxicity studies. The age of the animals would be chosen so that their relative stage of maturity at the start of dosing is equivalent to that of the youngest child that will be vaccinated. If, for instance, the vaccine is intended for use in children of two years and older, weanling rabbits could be used for the repeat dose study. The animals would then be mature by the end of the study (at least in the recovery group) so all of the usual endpoints can be determined. If the vaccine is intended for use in babies, suckling animals will have to be used. This may necessitate a prolongation of the study to allow the animals to reach sexual maturity before the terminal histopathological examinations. It is technically feasible to treat juvenile rodents or rabbits from the first week of age (Barrow, 2007), though intramuscular injections are difficult in very young rodents due to their low muscle mass.

9. Developmental immunotoxicity

Vaccines have been identified as a class of drugs likely to cause immunotoxicity (House & Selgrade, 2010). It seems reasonable to pay particular attention to possible adverse effects of vaccines on the development of the immune organs, even though developmental immunotoxicity investigations are not currently required for conventional pharmaceuticals (Barrow, 2009). Several chemicals, including a few drugs (e.g. diazepam, diethylstilbestrol and dexamethasone) have been shown to interfere with immune development (Holladay,

1999; Dietert & DeWitt, 2010). Both prenatal and postnatal exposure have the potential to cause developmental immunotoxicity (Leibnitz, 2005). Unlike most organs, the immune system only reaches maturity at around the time of adulthood, which renders it uniquely susceptible to postnatal insult. The consequences of developmental immunotoxicity include persistent immune depression (Allais, Condevaux, Fant, & Barrow, 2009) or a skewed TH1/TH2 balance (Dietert & DeWitt, 2010). Further to the possibility of increased susceptibility to infection, infestation or cancer, additional concerns include allergy (e.g. asthma) and autoimmunity (Burns-Naas et al., 2008). In practice, methods are currently available only to detect immune depression within the context of nonclinical developmental safety testing (Barrow & Ravel, 2005).

A basic battery of investigations could be incorporated, for instance, into the RDS/juvenile study proposed above. If the immune function is found to be intact in the adult animal at the end of the study, it can be assumed that postnatal vaccination did not adversely influence immune development. An initial assessment of the integrity of the immune system is provided by the routine parameters in the RDS study, i.e. clinical pathology (e.g. white blood cell counts), organ weights and histopathology of the immune organs (e.g. spleen, thymus, lymph nodes, liver and kidneys). An evaluation of lymphocyte subsets may also be a useful addition. In the case of specific concerns, functional tests, such as a primary antibody test to sheep red blood cells (White, Musgrove, & Brown, 2010) or a humoral response to keyhole limpet haemocyanin (KLH) (Plitnick & Herzyk, 2010) may also be performed. It must be noted, however, that most methods of immune evaluation have yet to be validated in the rabbit. One evaluation of immune function is already inbuilt in the proposed RDS/juvenile study, i.e. the ability of the animal to raise an immune response to the administered vaccine.

10. Fertility investigations

Fertility studies are not currently required for the approval of new vaccines. The current developmental toxicity protocol is not designed to detect effects on fertility. Anti-fertility effects in the female may be detected in the routine developmental toxicity study following the vaccination of the females before mating, but the study design is not well adapted for that purpose. It is not unusual to find a transitory poor mating performance in the vaccinated females compared with the controls. This is not entirely surprising, given the massive immune response that has been deliberately induced in the treated animals. Generally, delayed copulation under these conditions is not considered to constitute an adverse reaction, provided that the females eventually mate and prove to be fertile.

Vaccines may have the potential to induce infertility in both males and females by eliciting an immune reaction against hormones or components of the sperm or egg. Indeed, antifertility vaccines have long been proposed for use as contraceptives (Roitt, 2002). Any toxic effect of a vaccine that results in death of the preimplantation embryo would be manifest in a conventional nonclinical animal study as increased preimplantation loss. Such an effect may be caused by a direct interaction of the toxic agent with the conceptus or by neutralisation of the maternal hormones needed for the maintenance of pregnancy.

The preimplantation embryo may be uniquely sensitive to possible adverse effects of vaccines, since it does not benefit from the protection of the placenta barrier. During this stage, the embryo consists essentially of a ball of cells bathed in the intra uterine or fallopian fluid, with no protection against any antibodies, activated immune cells, cytokines or other bioactive molecules that may be present. Dysmorphogenesis (i.e. teratogenicity) due to exposure to a chemical before implantation of the embryo is very rare. The consequence of toxic insult on the undifferentiated embryo depends on the number

of cells that are destroyed; the embryo generally either dies or manages to repair the damage to develop normally (Hood, 2006).

The RDS studies include a histopathological examination that would be expected to reveal any direct effects on the reproductive organs (Forster, this issue). Mating studies are necessary to detect effects on libido or functional mating behaviour.

Mating studies are not practicable in monkeys (Chellman et al., 2009), so the assessment of male and female fertility in primate species is limited to the histopathological examination of the reproductive organs in the RDS studies.

11. Pharmacovigilance

The nonclinical studies described above are intended to ensure an optimum level of safety for new vaccines in proportion with the perceived hazards. We cannot, however, rely on these studies alone to be sure of detecting all possible adverse effects in the human population, in view of the known limitations of animal models for the detection of developmental toxicity, the limited nonclinical examinations performed and the relatively low number of animals used. Pharmacovigilance is essential, so that potential concerns in the human population can be identified and evaluated as rapidly as possible.

The Vaccines and Medications in Pregnancy Surveillance System (VAMPSS) will monitor the effects of seasonal and H1N1 flu vaccines given to pregnant women in North America (OTIS, 2010). The programme is coordinated by the Organization of Teratology Information Specialists and will run for the next seven years. Hopefully, the results of this project, along with others in other countries, will contribute to our understanding of the influences of vaccines on the course and outcome of pregnancy.

12. Conclusions

According to the available information, the undisputed benefits of vaccines far outweigh any potential effects on human development and reproductive performance. Nonetheless, theoretical mechanisms do exist by which vaccines could influence the course and outcome of pregnancy or have effects on fertility or juvenile development. The FDA guidelines on the preclinical testing of preventative and therapeutic vaccines for developmental toxicity brought significant improvements to the preclinical testing requirements for vaccines. Improvements in the methods and techniques for the detection of juvenile toxicity, developmental immunotoxicity and fertility effects are likely to be incorporated into the regulatory requirements in the future.

Acknowledgements

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