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New adjuvanted vaccines in pregnancy: what is known about their safety?

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The recent introduction of oil-in-water emulsions as adjuvants in several pandemic vaccines, such as the H1N1 vaccine, has challenged regulatory authorities to establish their safety in the general population, as well as in specific populations. Pregnant women were advised to be a target group for H1N1 vaccination owing to the risk of this group developing serious complications with this infection. However, the addition of adjuvants to the H1N1 vaccine has initiated a discussion on the safety of adjuvanted vaccines in this special population. Changes in the maternal immune system are essential for acceptance of the fetus and for development of the placenta. The potential effects on pregnancy of interfering with this uniquely adapted immune balance through the induction of proinflammatory reactions such as those induced by adjuvanted vaccines have only been studied rarely. Here, we review the available information and discuss how vaccination may interfere with pregnancy, fetal development and pregnancy outcomes.

KEYWORDS: adjuvant • decidual immunology • immunology • pregnancy • safety • vaccination

The vast majority of vaccines are not indicated for use during pregnancy and vaccination during pregnancy is generally avoided. However, a recommendation to vaccinate against seasonal influenza in pregnancy has become common in most countries in Europe and in the USA. During pregnancy, the risk of complications or hospitalization due to influenza infection increases and is highest in the third trimester [1,2]. A benefit/risk analysis suggests that influenza vaccination in healthy pregnant women in the second or third trimester and those with comorbidities in any trimester may be warranted [2–4]. This opinion is, however, not undisputed [5].

Thus far, no study has demonstrated an increased risk of maternal complications or adverse fetal outcomes associated with inactivated (seasonal) influenza vaccines as recently reviewed by Naleway [6]. However, in view of the pandemic threat of H1N1, several vaccine manufacturers decided to add an adjuvant to their H1N1 vaccines. This reduces the amount of antigen needed per vaccine to elicit an adequate immune response and thus increases the number of vaccines that could be produced at the time of a pandemic outbreak. The addition of such immunopotentiators to vaccines

intended for mass vaccination campaigns has initiated a discussion on the safety of the use of adjuvants in vaccines that are administered to pregnant women.

Owing to the fact that pregnant women are usually excluded from the majority of clinical trials on vaccines, little is known about the side effects of adjuvants during pregnancy. There are only a few published studies on the safety of adjuvanted vaccines during pregnancy [7–9]. These studies, however, concentrated on the extreme effects of adjuvanted vaccines on pregnancy (i.e., teratogenic effects). No attention has been paid to more subtle effects, such as effects on placental development or fetal weight or the effects of the vaccine or its adjuvant on the maternal immune system. During normal pregnancy, the maternal immune system adapts to accommodate the semiallogeneic fetus and interference with this immune system may interfere with normal pregnancy.

In this article, we will evaluate the changes that occur in the maternal immune system during pregnancy and discuss the possible effects of vaccines and, in particular, the effects of their adjuvants on the immune system and how this may interfere with pregnancy, fetal development and pregnancy outcome.

Pregnancy & the immune system

The maternal immune system during pregnancy is altered to actively tolerate the semiallogeneic fetus. These alterations include changes in local immune responses, that is, in the uterine mucosa (decidua) [10], and changes in peripheral immune responses [11]. After implantation, the uterine endometrium is rapidly infiltrated by fetal trophoblast cells; the endometrium will then develop into the decidua and ensure anchorage of the placenta and therefore proper fetal nutrition. However, this invasion needs to be properly regulated to protect the corporal integrity of the uterine wall of the mother. Both shallow- and over-invasion will lead to problematic pregnancies [12,13]. Local decidual immune cells, such as uterine natural killer (uNK) cells and macrophages, are important regulators of this balance between tolerance of fetal trophoblasts and limitation of their invasion [14,15]. When placental circulation is established, the peripheral blood also comes into close contact with fetal cells, specifically, villous trophoblasts. This may affect the peripheral maternal immune response.

Decidual immune responses

The decidua is an immunologically active tissue that is infiltrated by large numbers of maternal immune cells. The numbers of the different maternal immune cells change throughout the course of pregnancy [10,16,17]. The first trimester is dominated by uNK cells and alternatively activated macrophages [16,18–20]. The uNK cells are immunomodulatory but noncytotoxic in nature and, together with macrophages, have been suggested to be crucial for regulating trophoblast invasion and spiral artery remodeling [21,22]. Both uNK cells and macrophages decrease during the second trimester [17]. Although uNK cells and macrophages are the most dominant cells in the decidua, helper and cytotoxic T lymphocytes, as well as regulatory T cells, can also be found in the decidua [17,23–28]. They may also play an important immunoregulatory role. More recently, dendritic cells (DCs) have been shown to be present in the decidua [29,30–34]. The exact role of these cells in the decidua remains to be established, but they have been shown to promote a type 2 dominant state [34] and may be involved in immunotolerance induction.

Together these immune cells in the decidua play an important role in the acceptance of the semiallogeneic fetus, implantation and placentation. They do so by producing many factors, including cytokines and angiogenic factors that are able to regulate implantation of the blastocyst and placentation. A critical balance in the number of immune cells, as well as in the factors they produce, is extremely important for pregnancy and it has been shown that interfering with these local immune responses may result in defective placentation or pregnancy loss [35–37].

Changes in the peripheral immune response during pregnancy

During weeks 8–12, when placental circulation is established, the maternal peripheral blood is in close contact with semiallogeneic villous trophoblasts. These trophoblasts are able to produce and shed factors, such as IL-4 [38] and syncytiotrophoblast microfragments [38] or fetal cells [39,40] into the maternal circulation.

Such factors affect the immune system. The presence of pro-inflammatory factors in the plasma of pregnant women has been shown by Faas *et al.*, who demonstrated that incubation of monocytes with plasma from pregnant women activated this cell type [41]. Moreover, the passage of maternal blood through the placenta activates inflammatory cells such as granulocytes and monocytes [42]. The most important changes in the immune response in the maternal circulation during human pregnancy will be described later.

Innate immunity

It is generally accepted that the innate immune system is activated during pregnancy. The numbers of monocytes and granulocytes significantly increase during normal pregnancy [43–45] and these cells also show phenotypical and functional activation [11,44,46,47]. DC numbers, on the other hand, were found to be decreased during pregnancy [48–50] and, more recently, the functional properties of DCs have been studied. It was shown that expression of tolerance-inducing molecules, such as the costimulatory B7 complex [51] and indoleamine-2,3 dioxygenase, is increased in DCs during pregnancy [52]. Furthermore, the number of peripheral natural killer (NK) cells and their production of IFN- γ is decreased in pregnant women as compared with nonpregnant women [35,53,54] and it was also shown that the ratio of NK1/NK2 cells is decreased [55]. Such changes in NK cell populations are important for normal pregnancy, as demonstrated when Beer *et al.* showed that in an *in vitro* fertilization population, no live infants were born when the percentage of maternal peripheral NK cells was above 18% [56].

Specific T-cell-mediated immunity

The concept that a healthy pregnancy is accompanied by a decreased Th1/Th2 balance proposed by Wegmann *et al.* [57] has been confirmed by many others [35,58–60]. However, it is now recognized that although the Th1/Th2 balance is important in pregnancy, the immunological paradox of pregnancy is much more complicated. Th17 cells and regulatory T cells have also been shown to be involved in the complex immune regulation seen during pregnancy [61]. Various studies have recently demonstrated that regulatory T cells are essential in pregnancy for promoting immune tolerance [24,26,62]. Indeed, in peripheral blood, regulatory T cells are increased during early pregnancy [24,63] and in mice they have been shown to be required for the maternal immune system to tolerate a fetal allograft [62].

In addition, rather than having only deleterious effects on pregnancy, the type 1 cytokines were also found to be essential. It was shown that they are needed in triggering pregnancy-induced spiral artery remodeling [64–68].

Consequences of the adapted immune response for pregnancy

Although the maternal immune response changes during pregnancy, most pregnant women experience a healthy pregnancy, suggesting that the immunological changes do not dramatically affect the integrity of the mother. However, it has been shown

that pregnant women are more sensitive to certain infections. For instance, the risk of developing clinical disease after infection with poliovirus or hepatitis A virus is increased in pregnant women [69]. In addition, pregnancy has been shown to increase the infectivity of cytomegalovirus [69], herpes simplex virus [70] and malaria [71]. The changes in maternal immune responses are also reflected by changes in autoimmune diseases: rheumatoid arthritis often ameliorates during pregnancy, while systemic lupus erythematosus can flare up [72,73].

The altered immune responses seen during pregnancy are also apparent from the fact that pregnant animals are more sensitive to proinflammatory stimuli than nonpregnant animals [74–78]. This altered sensitivity may be particularly relevant for adjuvanted vaccines because adjuvants are usually potent proinflammatory stimulators. Pregnant animals are extremely sensitive to lipopolysaccharides (LPS), which is a potent stimulator of innate immune responses. In accordance with this, we have shown that infusion of an extremely low dose of LPS into pregnant rats induces hypertension and proteinuria [79], which are symptoms of preeclampsia, whereas infusions into nonpregnant animals did not [80]. It may be important to note here that the adjuvant monophosphoryl lipid A (MPL; AS04) is a less toxic but still an immunoreactive derivative of the endotoxin LPS [81,82]. Adverse effects on pregnancy have also been described after infusion of other proinflammatory substances such as extracellular ATP [83], poly I:C [84] and TNF- α [85], as well as injection of type 1 cytokines [86].

Taken together, the immune system significantly changes during pregnancy and these changes are essential for normal placental and maintenance of a healthy pregnancy. Interfering with the maternal immune system could disturb the newly formed balance between tolerance and immunity during pregnancy and may affect placentation, the outcome and/or the course of pregnancy. This raises questions on the safety of vaccination during pregnancy, especially with vaccines that have a strong immune-stimulating/modulating ability, for example, adjuvanted vaccines.

The use of adjuvants in vaccines

Initially, vaccines consisted of live-attenuated or inactivated whole-cell bacteria and whole viruses. However, as several of these vaccines were associated with side effects, a shift towards the use of highly purified proteins has been made to improve vaccine safety. As a consequence, the immunogenicity of some of these newly developed vaccines was reduced. This is assumed to be due to the absence of the pathogen's endogenous immune stimulators, since highly purified proteins are generally weak antigens [87,88]. To compensate for the reduced immunity, adjuvants can be added to the vaccine formulation.

An adjuvant can be defined as a substance that, when administered in combination with a specific antigen, enhances the immune response against the antigen when compared with the antigen alone. Adjuvants were first described in the 1920s [89,90] and have been used for decades to improve the immune response to vaccine antigens. Aluminium salts are the most widely accepted and used adjuvants [88,91,92]. Other types of

adjuvants are emulsions (water-in-oil or oil-in-water), liposomes and substances containing pathogen-associated molecular patterns [87,93,94] such as MPL. For an overview of adjuvants that are constituent of EU-registered vaccines, see TABLE 1. Additive or even synergistic effects have also been obtained with combinations of different types of adjuvants (e.g., in the human papillomavirus [HPV] vaccine Cervarix[®] [GSK Biologicals, Rixensart, Belgium], the adjuvant MPL is combined with aluminium hydroxide) [201]. In addition to the adjuvants that are part of a registered vaccine, there are also numerous other adjuvants under development; these include CpG motifs, immune-stimulating complexes (ISCOMs), microbial derivatives/Toll-like receptor (TLR) activators and several others [87].

The molecular mechanism of the majority of adjuvants is still only partially characterized. Adjuvants are generally thought to act by nonspecific stimulation of the innate immune system through targeting of 'professional' antigen-presenting cells (APCs) such as DCs [87,95–97]. DCs can capture, process and present (foreign) antigen in their major histocompatibility complex (MHC) molecules, which together with the appropriate costimulatory molecules can lead to the activation of naive helper and cytotoxic T lymphocyte cells. This activation of the adaptive immune system is needed for the protective effect of vaccination [97]. The mechanism by which the APCs are activated is important in the understanding of the molecular mechanism of an adjuvant. For some adjuvants, specifically the pathogen-associated molecular pattern-containing adjuvants, specific receptors on the APC have been identified, for example, TLR9 for synthetic oligodeoxynucleotides containing unmethylated CpG motifs or TLR4 for MPL [98,99]. However, for other adjuvants, knowledge about the mechanism of action is still limited. Even for aluminium salts, there is still controversy on the mode of action and several mechanisms have been postulated (for a review, see [92]). Many years ago, it was suggested that aluminium salts induce an antigen depot effect; however, other mechanisms have recently been described, such as recruitment and activation of immune cells, enhancing of monocyte differentiation into DCs and augmentation of antigen uptake and migration of DCs into the draining lymph nodes [92,100,101]. Similar mechanisms have also been postulated for other adjuvants (e.g., the oil-in-water emulsion MF59 and ISCOM/ISCOMATRIX[®] [CSL Behring, King of Prussia, PA, USA]) [100–102].

In addition to enhancing the immune response towards an antigen, an adjuvant may also steer the immune response in a certain direction. Aluminium, for instance, is a well-known Th2-promoting adjuvant [92]. For CpG motifs, it is known that they stimulate human B cells and plasmacytoid DCs, thereby promoting the production of Th1 and proinflammatory cytokines and the maturation/activation of professional APCs [103]. In addition, the saponin-based adjuvants such as ISCOMATRIX are more prone to induce Th1-directed responses [101,104]. The data on the modulation of immune responses of oil-in-water emulsions such as the EU-licensed adjuvant MF59 are less clear and not fully consistent. For MF59, it has been shown that one dose of MF59 H5N1 vaccine induces the expansion of CD4⁺ T lymphocytes with a Th1-prone effector/memory phenotype [105], while other

Table 1. Overview of adjuvants that are part of EU-registered vaccines.

Adjuvant	Composition	Vaccine	Antigen
AS03	Squalene, DL- α -tocopherol and polysorbate 80	Pandemrix® (GSK Biologicals, Rixensart, Belgium) Prepandrix® (GSK Biologicals) Arepanrix® (GSK Biologicals)	Influenza Influenza Influenza
MF59	Squalene, polysorbate 80 and sorbitan triolate	Fluad® (Chiron Vaccines, Siena, Italy) Aflunov®/Focetria® (Novartis, Siena, Italy)	Influenza Influenza
AS04	MPL and aluminium hydroxide/phosphate	Cervarix® (GSK Biologicals) FENDrix® (GSK Biologicals)	HPV Hepatitis B
AF03	Squalene, sorbitan oleate, polyethylene cetostearyl ether and mannitol	Humenza® (Sanofi Pasteur SA, Lyon, France)	Influenza
Aluminium	Aluminium hydroxide or aluminium phosphate or aluminium hydroxyphosphate sulfate	Ambirix® (GSK Biologicals) Daronix® (GSK Biologicals) Fluarix® GSK Biologicals) Gardasil® (Sanofi Pasteur MSD, Lyon, France)/Silgard® (Merck Sharp & Dohme Ltd., Hertfordshire, UK) HBvaxPRO® (Sanofi Pasteur MSD) Hexavac® (Sanofi Pasteur MSD) Infanrix® hexa (GSK Biologicals) Infanrix® penta (GSK Biologicals) Synflorix® (GSK Biologicals)	Hepatitis A and hepatitis B Influenza Influenza HPV Hepatitis B Diphtheria, tetanus, pertussis, hepatitis B, polio virus, <i>Haemophilus influenzae</i> type b Diphtheria, tetanus, pertussis, hepatitis B, polio virus, <i>Haemophilus influenzae</i> type b Diphtheria, tetanus, pertussis, hepatitis B, polio virus Pneumococcal polysaccharide

GSK: GlaxoSmithKline; HPV: Human papillomavirus. All registered vaccines in this table can be found at [209].

results suggest that MF59 stimulates the Th2 response [106–108]. Based on studies of MF59 combined with other adjuvants, it was postulated that MF59 amplifies the ongoing immune response but does not change the Th1/Th2 balance [109]. Data on the effects of other EU-licensed adjuvants on the Th1/Th2 balance, such as AS03 and AF03, are scarce in this respect.

Thus, adjuvants are stimulators of the innate immune response. As the innate immune system is activated during pregnancy, it may be suggested that an adjuvant may elicit a different, for example a stronger, immune response in pregnant women as compared with nonpregnant women or males. Such a stronger immune response may interfere with the newly formed pregnancy-induced balance of the immune response and thus interfere with pregnancy. Adjuvants may also interfere with the Th1/Th2 balance. Although pregnancy can be considered a Th2-dependent process [35,57–60], type 1 cytokines are also important for normal implantation and placentation. As adjuvants activate the immune response via different mechanisms and may steer the immune response towards Th1 or Th2 responses, it is currently not possible to predict which adjuvants can or cannot be safely used in pregnancy.

Vaccination during pregnancy

Information on the safety of several vaccines during human pregnancy can be found in public literature. Most data available concern the use of unadjuvanted seasonal influenza vaccines because

vaccination against seasonal influenza during pregnancy is recommended in several countries. No study to date has demonstrated an increased risk of either maternal complications or adverse fetal outcomes associated with inactivated, unadjuvanted (seasonal) influenza vaccination [4,6,110,111]. Therefore, vaccination against seasonal influenza is considered safe.

There are also data available from the WHO/UNICEF program against maternal and neonatal tetanus. Many pregnant women have received an alum-adsorbed tetanus toxoid vaccine in this program and thus far no association with fetal malformations has been demonstrated [112]. Although reports are scarce, it is likely that adverse effects by tetanus toxoid vaccines would have been reported when observed. Therefore, it is generally assumed that tetanus vaccination is not associated with major birth defects or severe adverse pregnancy outcome. Reports on the safety of other vaccines, including smallpox [113], polio [114], yellow fever [115], pertussis [7,8] and even anthrax [116] during pregnancy are reassuring and show a lack of association between maternal vaccination (including first-trimester exposure) and preterm delivery or major malformations. However, the studies generally focus on major malformations and most are not sufficiently powered to detect a moderate increased risk of birth defects. Therefore, more subtle effects such as early pregnancy loss, abortion, preeclampsia or reduced fetal growth may have been missed as potential adverse effects caused by vaccination

during early pregnancy. Overall, for the unadjuvanted seasonal influenza vaccines and tetanus toxoid vaccines, routine vaccination during pregnancy may be considered safe, while currently, for other vaccines, the limited data do not show side effects of the vaccination.

However, these observations may not be extended to all vaccines. Since it is the modulation of the maternal immune system that may affect pregnancy outcome, differences in the immune response to vaccines may have varying effects on pregnancy. Unadjuvanted seasonal influenza vaccines are relatively poorly immunogenic and only mildly activate the immune system when compared with adjuvanted influenza vaccines [117,118]. Therefore, the relevance of the safety data obtained with unadjuvanted (seasonal) vaccines in the assessment of the safety of adjuvanted (pandemic) influenza vaccines is very limited. In addition, the data obtained from the alum-adjuvanted tetanus vaccine are of limited relevance to the safety of the more recent oil-in-water adjuvant emulsions such as AS03 and MF59, which were used in the pandemic influenza vaccines, because of the differences in the immunomodulatory actions of these adjuvants.

There are a few publications of the pregnancy registries of the MPL/alum-adjuvanted HPV vaccine Cervarix [119,120,201]. Preliminary results of the analysis of these registries do not indicate that there is a significant effect of vaccination on fetal malformations or spontaneous abortions. However, the majority of uncertainty surrounds the effect of vaccination in women becoming pregnant within 30 days of vaccination, because of the limited amount of data [120,121].

There is only one publication on exposure to MF59-adjuvanted influenza vaccine during pregnancy [9]. A retrospective analysis of inadvertent pregnancies occurring in clinical trials by Novartis revealed that pregnancy outcomes in subjects exposed to MF59-adjuvanted influenza vaccine ($n = 43$) were similar to those exposed to unadjuvanted influenza vaccines ($n = 60$) at any time during pregnancy and also when exposure occurred in early pregnancy. These data are too limited to draw any conclusions on the safety of oil-in-water adjuvants during pregnancy. Therefore, data obtained in animal reproductive toxicity studies on the new adjuvanted vaccines are needed for the assessment of their safety.

Regulatory requests for reproduction toxicity testing of vaccines

The 2005 EMA guidelines on adjuvants [202] only provide guidance on when to perform reproductive toxicity studies, but specific guidance on the study design is missing. In the US FDA Guidelines *Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications* [203] it is recommended to optimize maternal antibody titers throughout the embryonic, fetal and early post-natal period, to ensure a peak antibody response during the critical phases of pregnancy (i.e., organogenesis) and to administer one or several doses during organogenesis. These recommendations are based upon the hypothesis that the risk of vaccination is associated with the increase of antibodies against the relevant antigens or with potential direct embryotoxic effects of the vaccine formulation.

As a result of these recommendations, many vaccine reproductive toxicity studies encompass one or several vaccinations prior to mating (to ensure the high antibody titers during pregnancy) and several vaccinations after implantation, during organogenesis. However, it is clear that in humans, antibodies are rarely transferred to the embryo and not at all in the first trimester because of the absence of neonatal Fc receptors [122]. It is only during the second trimester that there is an increase in placental transfer of antibodies, with a steady rise to maternal levels at the end of pregnancy [123]. The same is true for several animal species that have been used as models for vaccine testing [122]. Therefore, the risk to the fetus of high antibody levels during the first trimester (implantation and organogenesis) appears almost negligible.

Adverse effects on neonatal development and/or pregnancy may also be caused by more secondary effects of vaccination, such as the activation of the maternal immune system. As described previously, it is clear that specific changes in the maternal immune system occur during pregnancy and are necessary for implantation, placentation and a healthy course of the pregnancy. Activation of the maternal immune system may interfere with the necessary changes of this system for the maintenance of a pregnancy and could therefore affect the course and outcome of a pregnancy. As described earlier, the issue of interference of adjuvanted vaccines with the immune response of pregnancy has not been addressed in the FDA's guideline on reproductive toxicity of vaccines, or in the WHO's 2005 Guideline on Preclinical Evaluation of Vaccines [204].

Reproductive safety testing of adjuvants or adjuvanted vaccines

Publicly available data on nonclinical reproductive safety testing of adjuvants/adjuvanted vaccines are very limited. Most information can be found in the European public assessment reports (EPAR) of EU-registered vaccines that contain an adjuvant (TABLE 1).

The EPAR of the MF59-adjuvanted influenza vaccine Focetria® (Novartis Vaccines and Diagnostics, Siena, Italy) shows that MF59 was tested for reproduction toxicity in two rabbit studies and one rat study, either as single agent or in conjunction with influenza proteins or other antigens [205]. The two studies using rabbits investigated the effects of vaccination on either day 6 through to day 28 of gestation, or three times before mating and at days 7 and 20 of gestation. Information on the timing of dosing in the rat study was not described. In these studies, MF59-adjuvanted vaccines were well-tolerated, did not cause maternal or embryo–fetal toxicity, were not teratogenic and had no effects on post-natal development. It was concluded that there are no specific concerns for the use of this adjuvanted vaccine in pregnancy.

The AS03 adjuvant is present in two pandemic influenza vaccines, Pandemrix® (GSK Biologicals) and Arepanrix® (GSK Biologicals). The potential reproductive toxicity of this adjuvant was tested in rats, with and without influenza antigen [206,207]. For both products, animals were dosed once before mating and at days 6, 8, 11 and 15 of gestation for Pandemrix and at days 7,

9, 12 and 16 for Arepanrix. The only potential treatment-related effect was a somewhat delayed air-righting reflex for some of the offspring of AS03-treated dams in the Pandemrix study.

Another pandemic influenza vaccine, Humenza® (Sanofi Pasteur, Lyon, France) contains the adjuvant AF03. Two studies in rats and rabbits have been reported using the adjuvant alone. Two rabbit studies using the AF03-adjuvanted A/California/07/2009 H1N1 influenza vaccine have been conducted with a similar schedule as for Pandemrix (days -21, -7, 6, 8, 11 and 27) and no embryo–fetal toxicity effects have been noted [208].

AS04 is another EU-registered adjuvant present in an HPV vaccine (Cervarix) as well as in a Hepatitis B vaccine (Fendrix® [GSK Biologicals]). For this adjuvant, no effect on any of the phases of reproduction or fetal development was seen in a reproductive toxicity study in rats [201]. However, no data on the timing of vaccination during pregnancy were provided.

There are multiple registered vaccines that contain aluminium salts as adjuvants (TABLE 1). Reproductive toxicity studies have not been performed with most of these vaccines, either because the ingredients and their concentrations are within ranges of already marketed or nationally registered vaccines or because the vaccine is not intended to be used in women of childbearing potential. For those vaccines that were tested (Gardasil® [Sanofi Pasteur MSD SNC, Lyon, France/Silgard® [Merck Sharp and Dohme Ltd., Hertfordshire, UK]), information on study design was lacking, but no adverse effects were reported.

A review of these studies confirms the observation that vaccine administration around the implantation phase of the embryos is generally not included in the study designs, while only booster vaccinations and not primary vaccinations are mostly given during pregnancy. These booster vaccinations are generally given at the time of implantation or after this. Thus, potential adverse effects due to vaccination early in pregnancy are not covered by the reproductive toxicity studies. According to the EPAR of Pandemrix, the marketing authorization holder has agreed to conduct a study in which the vaccine will be given to pregnant animals early in their pregnancy. The outcome of this study, when published, will be interesting in relation to this subject.

As noted earlier, low doses of proinflammatory components such as LPS, may have serious consequences during pregnancy, such as the development of preeclampsia at the end of pregnancy [80]. It is not clear from the EPAR reports whether end points reflecting such effects have been included in the developmental and reproductive toxicology studies. Another apparent feature of the study designs is that the animals are often pretreated long before mating, while this is not a reflection of the clinical situation when a vaccine is administered for the first time during pregnancy. This may have some consequence for the interpretation of the results because the immune response during a booster immunization is likely to be different from the response after a first administration [105].

Several other studies in which pregnant animals have been vaccinated with adjuvanted vaccines can be found in the public literature [124–128]. Adverse effects in these studies were not detected/reported, but the studies were generally small scale, aimed at detecting efficacy and not safety, and vaccination

was usually performed in the second half of pregnancy. In one study, pregnant rats received a single intraperitoneal injection of either Bacillus Calmette–Guérin, *Bordetella pertussis* or *Cryptosporidium parvum* vaccine at the day of implantation. In this study, the bacterial adjuvants were not abortogenic. However, a nonsignificant trend towards a larger fetoplacental interface compared with control was noted [129].

Taken together, even though the available animal data might be reassuring, several critical concerns, such as the safety of primary vaccination in the first trimester of pregnancy, have not been addressed during reproduction toxicity testing.

Discussion

Little is known about the safety of adjuvanted vaccines during pregnancy, particularly with regard to the recently introduced adjuvanted vaccines. It is slowly becoming recognized that exclusion of pregnant women from clinical trials withholds them from safe and effective treatment [130]. This is very true for vaccination during pregnancy, especially when using adjuvanted vaccines. The safety of adjuvanted vaccines in pregnant women has not been tested in clinical trials. A simple extrapolation of safety data of vaccines from the nonpregnant populations to the pregnant population is impossible owing to the changes occurring in the immune system during pregnancy. Proinflammatory stimuli that are present in vaccines, in particular in adjuvanted vaccines, may induce a stronger immune response in pregnant individuals, since pregnant individuals have been shown to be more sensitive to proinflammatory stimuli than nonpregnant individuals [74–78]. Moreover, vaccination may negatively affect the immune balance needed for a successful pregnancy in women. Consequently, vaccination may increase the risk of breaking immune tolerance against the fetus. This could theoretically result in pregnancy loss, restricted growth of the fetus and/or the placenta or in the development of preeclampsia.

Human data, thus far, suggest that there does not seem to be a particular concern with the use of vaccines during pregnancy. However, these data are mostly based on experience with unadjuvanted seasonal influenza vaccines and alum-adjuvanted tetanus vaccines, and their effects cannot simply be extrapolated to all vaccines. Human data on the safety of the more recently introduced adjuvants during pregnancy, that is, the oil-in-water emulsions MF59 and AS03 and the MPL/Alum combination of AS04, are limited. For oil-in-water adjuvanted vaccines, the limited human and animal data suggest that there are no direct teratogenic or other adverse effects when the vaccine is administered during the second and third trimester. The available safety data for the first trimester are too limited to draw any conclusions on the safety of the new adjuvanted vaccines in this period. In theory, a strong activation of the maternal immune system during this period, such as that induced by an adjuvanted vaccine, could have an impact on the implantation of the embryo and acceptance of the fetus.

In addition to the available human data, another important argument for claiming the safety of adjuvanted vaccines during pregnancy is that the immunological effects of adjuvants are local and that systemic effects are not expected. However, systemic

effects such as malaise, myalgia and fever have been reported after administration of adjuvanted influenza vaccine [131]. This indicates that vaccination is more than a local reaction and that systemic effects are also induced. The cause of the systemic effects following vaccination is unknown, but it is probably due to triggering of cytokines of the innate immune system, such as IL-1, IL-6 and TNF- α , which can function as endogenous pyrogens [132]. While it may be unlikely that circulating maternal cytokines reach the fetal circulation and affect the fetus directly [133,134], these cytokines may affect placentation [86] and may induce abortions [84,79] as seen, for example, in primates receiving human cytokines [135]. The effects of proinflammatory substances such as LPS on pregnancy may also relate to the induction of cytokine production and the effects of these cytokines on pregnancy [83–85]. Animal and human data addressing the proinflammatory effects of adjuvanted vaccines in pregnancy are thus far lacking.

Expert commentary

Pregnancy is a sensitive period with respect to the effects on the immune system and there are still many questions concerning the strength of the balance between tolerance and immunity. One of the questions is whether the immunological response following administration of a vaccine during pregnancy can disturb this balance. As it is generally accepted that seasonal influenza vaccinations are safe during pregnancy, it seems likely that weak immunogenic vaccines do not interfere with the immune response of pregnant women to a great extent. It is clear that the presence of adjuvants in vaccines changes the immune response to the antigen quantitatively, but possibly also qualitatively by changing the Th1/Th2 balance. Therefore, administration of an adjuvanted vaccine during pregnancy may, in principle, affect the course of the pregnancy. This potential risk should be taken into account when considering vaccinating a pregnant woman with an adjuvanted vaccine, especially during early pregnancy. Animal data addressing this concern are thus far insufficient because this issue has not been addressed in the design of reproductive toxicity studies. It is clear that, in the future development of vaccines, more attention should be paid to the potential effects of immune stimulation on the pregnancy, in particular during early pregnancy.

A decision on whether or not to administer a vaccine during pregnancy is not only based on knowledge of its adverse effects or its risks, but also on the beneficial effects of vaccination. This benefit of vaccination, in other words, protecting the mother and the fetus against serious infections that lead to even more serious disturbances in the immune system and hyperthermia, and consequent adverse effects on pregnancy, is important to keep in mind. The benefit–risk evaluation for seasonal influenza vaccination in pregnant women is positive, since pregnant women who contract influenza are at an increased risk of complications, while there do not appear to be any adverse effects of vaccination. In addition, for tetanus, the risks to the fetus of vaccination is considered to be less than the risk of neonatal tetanus.

For the vaccines with new adjuvant systems, such a benefit–risk evaluation is very difficult as the risk, albeit based on theoretical considerations, cannot really be estimated at this time. Despite this

limitation and based on a positive risk–benefit estimation, several governmental organizations during the recent H1N1 influenza pandemic decided to administer adjuvanted vaccines to pregnant women (Sweden, The Netherlands and Finland), although not in the first trimester (The Netherlands and Finland). However, in other countries the use of a nonadjuvanted vaccine was recommended during pregnancy (Celvapan® [Baxter AG, Vienna, Austria] in Germany and Panenza® [Sanofi Pasteur] in France). In our opinion, more data are needed to determine whether the benefits of the use of these vaccines and their adjuvants outweigh the risks of developing the potentially severe pregnancy complications, such as preeclampsia or even abortion. Therefore, until more information on the safety of the new adjuvanted vaccines has become available, for example, through the establishment of pregnancy registries for newly developed adjuvanted vaccines, we recommend that immunization with adjuvanted vaccines during pregnancy is best avoided. However, when (inadvertently) administered during pregnancy, invasive diagnostics or even termination of pregnancy is not needed because, thus far, there are no data showing major malformations or birth defects caused by vaccination during pregnancy.

Five-year view

In the coming years, epidemiological data on the safety of recently introduced vaccines will become available, especially for the H1N1 vaccine (the first analyses from the pregnancy registries that have been set up during the recent pandemic influenza mass vaccination campaigns are expected in early 2011). It is extremely important to use these data to increase our knowledge on the safety of adjuvanted vaccines during pregnancy. It will provide valuable information needed to estimate the risk of the use of such adjuvanted vaccines during pregnancy. This will aid the benefit–risk evaluation and may change the recommendations on the use of these vaccines during pregnancy. In addition to these clinical data, it is also important to perform animal studies on the safety of the adjuvanted vaccines, with special attention to the effects of primary vaccination and early pregnancy. We hope that the discussion on the safety of the use of adjuvanted vaccines may change the design of nonclinical reproductive toxicity testing of vaccines. We anticipate that more knowledge on the mechanisms of action of adjuvants, and of their effects on the Th1/Th2 balance in particular, will become available. This might also increase the confidence in the estimation of the risk of the use of adjuvanted vaccines during pregnancy. Moreover, more fundamental research on the function of the immune system in establishing and maintaining a successful pregnancy is ongoing, which will lead to a better understanding of the immunological paradox of pregnancy.

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Key issues

- During pregnancy, significant changes in the immune system occur that are essential for placentation and maintenance of a (healthy) pregnancy.
- Thus far, all the data indicate that vaccination during pregnancy may be relatively safe.
- Adjuvants are potent proinflammatory stimulators that have been used for decades to improve the immune response to a vaccine antigen.
- Little is known about the potential side effects of adjuvanted vaccination during pregnancy.
- Other potent proinflammatory stimuli, such as lipopolysaccharides, poly I:C and type 1 cytokines, affect pregnancy in experimental animals.
- The potential adverse effects of activating the immune system during the implantation (and placentation) period are not addressed in the design of most reproductive toxicity studies on the safety of vaccines.
- Many pregnant women were vaccinated with the adjuvanted H1N1 pandemic influenza vaccine at a time when the safety of their use during pregnancy may not have been sufficiently addressed. This was done because the expected benefit was bigger than the perceived risks.
- Analysis of pregnancy registries of the H1N1 vaccines will provide valuable data concerning the use of oil-in-water adjuvanted vaccines during pregnancy.

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