Review Article

An Interspecies Comparison of Placental Antibody Transfer: New Insights Into Developmental Toxicity Testing of Monoclonal Antibodies

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There are profound differences in maternofetal transfer of immunoglobulins between species with extensive gestational transfer of maternal immunoglobulins in primates (including humans) via the chorioallantoic placenta as well as in rabbits and guinea pigs via the inverted yolk sac splanchnopleure. In contrast, other neonatal rodents (rats and mice) receive passive immunity predominantly postnatally. This transfer is mediated principally via FcRn receptors. Therapeutic monoclonal antibodies (mAbs) are most commonly of the IgG1 subclass, which is transported most efficiently to the fetus. In all animal species used for testing developmental toxicity, fetal exposure to IgG is very low during organogenesis, but this increases during the latter half of gestation such that the neonate is born with an IgG1 concentration similar to the mother (but not rats and mice). Review of mAb developmental toxicity studies of licensed products reveals Cynomolgus monkey as the species used in the majority of the cases (10 out of 15). Pregnancy outcome data from women gestationally exposed to mAb is limited. In general, the findings are consistent with the expected low exposure during organogenesis. Guinea-pigs and rabbits are potential candidates as “alternatives” to the use of nonhuman primates as the maternofetal transfer in the last part of gestation is at a level similar in humans. Based on the pattern of placental transfer of IgG in humans, study designs that allow detection of both the indirect effects in early gestation plus the effects of direct fetal exposure in mid and late gestation are recommended for developmental toxicity of mAbs. Birth Defects Res (Part B) 86:328–344, 2009. © 2009 Wiley-Liss, Inc.

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INTRODUCTION

Since the mid 1990s, there has been increased use of antibody-based therapeutics (Carter, 2006). Recombinant antibody medicines are used in oncology, in body imaging diagnostics, for treatment of autoimmune diseases, for passive immunization in infections, to neutralize intoxications, and to prevent graft rejection in transplantation, among other uses (Mix et al., 2006).

Antibodies are naturally occurring proteins of the body and their protein structure is considered safe to the host or to the developing fetus. Furthermore, it is known that fetuses of many mammal species including human, passively obtain maternal antibodies via placental transfer (Englund et al., 2003). These antibodies protect neonates from severe infectious diseases during their first vulnerable months of life, when their immune system is not yet fully developed and functioning (Brent, 2003, 2006). However, therapeutic mAbs engineered as being directed against mammalian targets could be able to interrupt developmental processes.

Antibody-based therapeutics are increasingly intended for long-term use. This could include treatment of women in reproductive age and during pregnancy. Therefore, as antibody-based medicines can result in fetal exposure, concerns have arisen that therapeutic antibody administration could cause adverse developmental effects including miscarriages or birth defects (Henck et al., 1996).

The placental transfer of antibodies is regulated specifically in each species (Baintner, 2007). There are...
different mechanisms for uptake and transport of maternal antibodies.

There is limited experience of assessing reproductive/developmental toxicity of antibody medicines in experimental animals and of assessing the predictive value for the human application of those tests that have been performed. Not surprisingly, given the relatively short number of years of post-marketing experience, there is limited information available about the use of monoclonal antibodies in pregnant women.

This review is designed to provide insight into the placental differences in immunoglobulin placent transport and placental structure in the laboratory animal species most commonly used in developmental and reproductive toxicity (DART) testing of antibody-based therapeutics. It also provides an inventory of the preclinical DART studies with monoclonal antibodies (mAbs) submitted to EU regulators followed by the present experience in human pregnancies.

This review helps to evaluate the suitability of different animal models and study designs for examining the potential risks arising from human pregnancy exposure to these monoclonal antibodies. Finally, a view will be given on the impact of the results of this review on a more optimal design for DART studies in nonhuman primates.

PLACENTAL BARRIER DEVELOPMENT AND STRUCTURE

“The mammalian placenta is an organ formed by the fusion of maternally and embryonically derived tissues inside the uterus” (Faber and Thornburg, 1983). In placental tissue, the maternal and fetal micro-vessels are so close to each other that certain materials transported in blood are able to diffuse from one bloodstream to another. Fetal and maternal bloods do not mix, as they are separated by a thin layer of placental tissue. Placenta functions include secretion of hormones, maintenance of an immunological barrier, and appropriate metabolic exchanges between the mother and the embryo (Enders and Blankenship, 1999).

There are a number of reviews on the placental function and on comparisons between placental functions in several animal species and humans (Brambell, 1970; Faber and Thornburg, 1983). It is not the purpose of this review to redo all that excellent work, but rather to focus on the role of placental function in antibody transfer.

Two types of placental arrangements can be distinguished, which are involved in antibody transfer from mother to young during the gestation of the animal species covered in this review:

1. Vitelline placenta, or inverted yolk sac placenta, consists of two layers: fetally-vascularized mesenchyme as the inner layer and endodermic epithelium as the outer layer. Vitelline placenta can be found in mouse, rat, guinea pig, and rabbit. The inverted yolk sac placenta has an important role in the maternofetal exchange in these species (Leiser and Kaufmann, 1994; Carney et al., 2004). Brambell et al. (1949) could show that the rabbit inverted yolk sac transported antibodies to the fetus. In rodents, the inverted yolk sac placenta, developed in early pregnancy, is equipped with a large variety of transfer mechanisms. It is closely apposed to the uterine wall. This indicates that it has an important role in maternal-embryonic exchange in early postimplantation (Gestation Day [GD] 7 to ~12, mating is GD 0) rodent embryos. The visceral yolk sac is functional by GD 9 and remains functional until term, even after the development of chorioallantoic placenta (GD 11–12 for rat) (Beckman et al., 1990; Enders and Blankenship, 1999). The yolk sac also surrounds the embryo completely. Compared to rabbit and human, rodent embryo is surrounded by much smaller volumes of fluid within the visceral yolk sac (Enders and Blankenship, 1999). In rabbits, different from the rat, the yolk sac is not closely apposed to the uterus during early organogenesis. This structure starts to develop at GD 9, encloses the embryo completely relatively late in development (GD ~13), and persists until term. From that it could be concluded that the early rabbit yolk sac is not a very efficient transporter (Carney et al., 2004).

2. Chorioallantoic placenta. The allantois is an outgrowth of the hindgut of the embryo (endoderm). It is also known as the extra-embryonic urinary bladder. The highly vascularized mesenchyme around allantois fuses with the inner vascularized layer of chorion, forming chorioallantoic placenta, most commonly known as “the placenta.” The vessels vascularizing the allantois sac and later the chorioallantoic placenta become the umbilical cord vessels. The lumen of allantois diminishes in some species (human), whereas in other species, like in sheep, it maintains its size and keeps functioning as fetal bladder. The chorioallantoic placenta is the main organ of materno-fetal exchange in both human and nonhuman primates (Faber and Thornburg, 1983; Sadler, 2004).

Types of placentas can be differentiated based on their shape and degree of concentration of exchange tissue area. The species covered with this report have either bidiscoidal (Rhesus, Cynomolgus monkeys) or discoidal placental shape (all other species discussed hereby). Discoidal placenta has the most concentrated placental area, in the majority of cases formed from chorioallantoic placental tissue. Not only these placental regions but also the other smooth areas, also called paraplacenta, contribute to the materno-fetal exchange. In rodents, the yolk sac placenta represents the paraplacental part of the membranes (Leiser and Kaufmann, 1994).

Three distinct periods in the functional life of the yolk sac can be differentiated in the rabbit. In the early period up to GD8, maternal antibodies (and other plasma proteins) appear in the fluid of the primary yolk sac traversing the uterine lumen (Brambell, 1958). During the second period, GD8 to GD13, antibody transmission is minimal during the inversion process when the yolk sac undergoes morphological changes. During the last period, GD15 to GD32, the endodermal cells of the yolk sac splanchnopleure are in contact with the uterine epithelium (Brambell et al., 1949). Antibodies, when undergoing materno-fetal transfer, are escaping the capillaries of the uterine endometrium, diffusing over the uterine lumen, crossing the yolk sac, eventually entering the fetal circulation (Faber and Thornburg, 1983).
In early human placenta, on the contrary, the exchange tissue area (villi) is formed around the entire surface of the conceptus. This placental shape is called diffuse placenta. By the third month of pregnancy, only the villi near the initial site of implantation have persisted, leading to the formation of the disc-shaped placenta. Although chorioallantoic placenta in humans begins functioning already by the end of the fourth week of pregnancy, this process is completed with the formation of disk-shaped placenta (Enders and Blankenship, 1999; Sadler, 2004). Also, the work of Jauiaux and Gulbis (2000) indicates that the chorioallantoic placenta in humans does not function in very early pregnancy. During the first trimester, the human fetus is surrounded by two fluid cavities, i.e., the inner amniotic cavity and the outer extra-embryonic coelomic cavity. Biochemical analysis of the coelomic fluid has shown that this is an ultrafiltrate of the maternal serum with the addition of specific substances (Jauiaux et al., 1991).

The tissue layers separating maternal and fetal blood can differ in number as well as type of layers, and it has been hypothesized that the thickness of the placental exchange barrier and the number of tissue layers governed the antibody transfer (Grosser, 1927, cited by Faber and Thornburg, 1983). In hemochorial placenta, characteristic to the species covered in this review, the chorioallantoic placenta is only formed of fetal vessels’ endothelium and trophoblastic layer, bathed directly in the maternal blood (Van der Aa, 1998). The overview of placental characteristics of the species covered with this article is given in Table 1.

### IMMUNE SYSTEM ACTIVITY DURING PREGNANCY

Pregnancy affects both the innate and the adaptive arms of immune system in the body. There is an increase in the number and activation state of circulating monocytes and granulocytes, which leads to a more aggressive attack on invading bacteria. On the other hand, the function of T-cells and natural killer cells is suppressed, thus protecting the fetus from destruction by maternal immune response. Suppressed T-cell activation in pregnancy is associated with increased susceptibility to viral infections and with remission of T-cell-mediated autoimmune diseases, such as rheumatoid arthritis or multiple sclerosis, during pregnancy (Luppi, 2003; Østensen and Villiger, 2007; Saraste et al., 2007). At the same time, B-cell function and antibody production does not change during pregnancy, indicating that the maternal immune system is more likely to respond to a challenge with antibodies than with activated T-cells (Luppi, 2003).

The transmission of humoral immunity from mother to young was originally demonstrated on mice by Ehrlich in 1892 (for a review, see Brambell, 1970). Transmission of maternal antibodies to young can occur prenatally via placenta and/or postnatally via maternal milk and this process is known as passive immunity. Maternal antibodies protect neonates from infectious diseases, during their first months of life, when the immune system of a neonate is not yet able to produce efficient amounts of antibodies themselves (Brent, 2003, 2006). In the next sections we will provide an overview.

### MATERNOFETAL TRANSFER OF ANTIBODIES IN HUMANS

#### IgG Antibody (Sub) Classes Transferred

The human humoral system is constituted by five major classes of antibodies, but human placenta seems to be impermeable to all classes except IgG (Avrech et al., 1994; Mix et al., 2006). IgG in itself is a class of antibodies consisting of four subclasses: IgG1, IgG2, IgG3, and IgG4.

As most of the IgG in a fetus is of maternal origin, its concentration in the fetus reflects transport from the mother (Simister, 2003). Fetal IgG remains on a low level until the second trimester of pregnancy and starts to rise smoothly during gestation weeks (WG) 13–18. A sharp increase in total IgG levels is observed between 22 and 26 WG (Gitlin and Boesman, 1966; Gitlin and Biasucci, 1969). Total IgG in the fetus continues to rise during the last trimester and typically exceeds maternal IgG concentration levels at term. The rate of increase between 29 and 41 weeks is roughly twice that seen between 17 and 28 WG (Malek et al., 1996; Garty et al., 1994) (see Fig. 1).

Most of the excess in total IgG in fetal circulation at term is formed by IgG1. The levels of IgG1, IgG3, and IgG4 showed an exponential rise during pregnancy. There is general agreement that the IgG1 subclass is transported most effectively, followed by IgG4, IgG3, and finally IgG2 with the least transport. The fetal concentrations of IgG3 and IgG4 at term reached similar levels as in the maternal circulation. IgG2 levels, however, showed slow linear rise throughout gestation, remaining significantly lower (up to 60%) than the concentration in the maternal blood. The IgG2 levels in the fetus at 17–22 WG were three and at 37–41 WG seven times lower in comparison to IgG1 (Garty et al., 1994; Malek et al., 1996).

#### Placental Transport Mechanisms of IgG

Immunoglobulins, as large hydrophilic molecules with a molecular mass of approximately 150 kD, cannot be transported by simple diffusion. They require active transport across the placental barriers via specific receptor-mediated mechanism (Saji et al., 1999). IgG transport across placenta is found to be regulated by two cellular barriers: the syncytiotrophoblast and the fetal capillary endothelium (Van der Aa et al., 1998; Takizawa et al., 2005).

**Syncytiotrophoblast.** A first step of IgG materno-fetal transfer takes place via specific receptor-mediated binding (Malek, 2003). The receptor involved is called neonatal Fc receptor (FcRn), which comprises β2-microglobulin and a large subunit resembling the α-chains of major histocompatibility complex (MHC) class I molecules (Story et al., 1994). The Fc portion of IgG has been found to bind with high activity to FcRn at an acidic pH (<6.5), although not at a physiological pH (7.4) (Roopenian and Akilesh, 2007). A syncytiotrophoblast internalizes maternal IgG-containing fluid into the endosomes. These endosomes are then gradually acidified, which allows IgG to bind to the FcRn, present in this compartment. The vesicle fuses thereafter with the membrane on the fetal side of the syncytiotrophoblast, where the physiological pH promotes the dissociation of IgG from FcRn (Roopenian and Akilesh, 2007).
Several studies have shown IgG presence in tubulover- 
icular structures, the possible early endosomes, in the syncytiotrophoblast (Lin, 1980; Leach et al., 1991). The data clearly support the hypothesis that IgG is transcyto- 
osed with its receptor (Leach et al., 1991).

Besides carrying IgG through the membrane, binding with FcRn also protects IgG from lysosomal degradation. It has been found that FcRn receptor might be saturated at high concentrations of IgG, thereby providing an explanation for the observed increase in IgG elimination rates with increasing administered IgG concentrations (Lobo et al., 2004).

**Fetal capillary endothelium.** The role of the second placental barrier, the fetal capillary endothelium, is not yet clear. FcRn is only occasionally or weakly expressed on fetal vessel endothelium, suggesting there should be another mechanism involved in IgG transport across this placental layer (Saji et al., 1999). As the endothelium is quite tight, it is presumed that IgG crosses this by a transcellular route, rather than para-
cellular route between endothelium cells. It has been assumed that IgG moves passively via transcytosing caveolae. However, with human term placentas, Takiza-
wa et al. (2005) could not detect IgG present in those caveolae. Koyama et al. (1991) have also found the markedly increased transcription of the FcγRIIb gene at 
WG 20 in human, when maternal IgG transfer begins (Saji et al., 1999). It is, therefore, hypothesized that IgG binds to FcγRIIb and gets transported across the endothelium passively downwards the concentration gradient in association of the FcγRIIb organelles (Takiza-
wa et al., 2005).

However, there are conflicting data. Firstly, FcγRIIb has no detected affinity for monomeric IgG. Secondly, FcγRIIb has higher affinity for complexes of IgG3 rather than IgG4, opposite to the results of transport capacities of IgG subclasses, discussed above. Therefore, it remains to be decided whether FcγRIIb is involved in prevention of immune complexes transport into the fetal circulation or rather mediates transport of monomeric IgG across the placenta. (Simister, 2003).

Higher total maternal IgG concentrations have been found to cause decreased levels of both total and specific IgG in infants, which may be due to the saturation of IgG transfer. German mothers had about half of the quantity of total IgG in their serum at the time of delivery as 
compared to Nigerian mothers, probably due to their lower pathogen exposure. Despite that, German mothers transferred a higher amount of measles antibodies to 
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**Transport of IgG in Early Pregnancy**

During the first trimester of pregnancy, the FcRn-receptor on the placental syncytiotrophoblast is hardly detectable and has not been detected before WG 14 (Israel et al., 1993). Very low levels of maternal IgG are present in the villous stroma of placentas during the first trimester (WG 8–10) (Bright and Ockleford, 1995). Jauniaux et al. (1995) have reported measurable concentrations of specific IgG and IgA in coelomic fluid samples from 6 weeks of gestation. Also Gurevich et al. (2003) detected IgG transfer into embryonic tissue as early as week 4, but no real quantification has been given, although concentrations are supposed to be low. This suggests that there might be an alternative transfer mechanism for IgG transfer during very early pregnancy, but no specific mechanism has been identified thus far.

Less effective transport through placenta during the first and second trimester of pregnancy might be due to the presence of an additional barrier of the cytotropho-
blast. This layer gets initially broken only after the fourth 
month of pregnancy and becomes less prominent when the placenta matures. During the period between WG 15 and 25, also the IgG concentrations in amniotic liquid are at their highest level, reaching 40 mg/100 ml (2–4% of maternal level) (Cederqvist et al., 1978). IgG has been found in the meconium (mucus found inside fetal intestines) in the gastrointestinal tract already from WG 13. As mentioned above, Fc-receptors have also been found on the microvillous surface of the human fetal intestine but only from WG18. At the beginning of the fifth month, the fetus begins to swallow and fetal swallowing of amniotic fluid has been shown to be the major process involved in protein clearance from 
amniotic fluid. Evidence is provided that human fetuses in early gestation (WG 18) swallow the amniotic fluid, and IgG uptake from ingested fluid would be mediated by fetal intestinal FcRn receptors (Israel et al., 1993). The fetus is estimated to drink about 400 ml/day, half of the total amount of amniotic fluid (Sadler, 2004).

**MATERNOFETAL TRANSFER IN DIFFERENT ANIMAL SPECIES**

**Antibody Transfer During Gestation in Different Animal Species**

According to Baintner (2007), transmission of antibo-
dies can be seen as a dual process and includes maternal secretion (e.g., in breast milk) followed by the absorption by the fetus. Human and possibly also other primates are the exceptions, where the antibodies are transported directly across the fetal cell layers of the placenta, and this process is the topic of this review. Thus IgG transfer from mother to the young is demonstrated to take place prenatally in utero and/or postnatally with first maternal milk, depending on the mammal species. For a short overview, see Table 2.

As in humans, the mammalian Fc (FcRn, neonatal FcR) receptors for native IgG have a key role. The α-chains of the FcRn and MHC class I molecules are considerably homologous in different mammalian species and hu-
mans and bind to identical β2-microglobulin light chains (e.g., for rats: Hunziker and Kraehenbuhl, 1998). The β2-
microglobulin is needed for translocation of the receptor from the cell surface to the interior (Baintner, 2007).

FcRn receptor is actually found in many tissues and at all ages, despite its name as a “neonatal” receptor. However, the expression levels are different. As the focus of this review is IgG transfer, the FcRn role only in IgG transport is discussed.

These receptors can be divided into two subclasses, depending on the environment pH they require for functioning. There are acid-dependent and -independent types of neonatal Fc receptors. Both receptor types bind IgG in a slightly acidic environment (pH 5–7), whereas
acid-dependent receptors do not function at the neutral or slightly alkaline pH of tissue fluids (pH 7.4). Acid-dependent receptors are found in the acidic environment of proximal small intestine of suckling mammals and also in the vesicles, vacuoles, and endosomes in different cell types (Baintner, 2007).

The mammalian FcRn receptor from a certain species (e.g., rabbit) binds IgG derived from other species with very different affinities, resulting in a different degree of yolk sac–mediated transfer. Brambell et al. (1950) already showed that homologous IgG is preferred over heterologous (e.g., equine and bovine) IgG, which was later confirmed (Batty et al., 1954; Tsay et al., 1980). There appears to be an important difference between binding and transfer, as human IgG showed only 10% relative binding (Batty et al., 1954), while exerting 80% transfer (Tsay et al., 1980), which is an important feature when thinking about testing monoclonal antibodies in rabbits. Similar species specificity has been described for the guinea pig (Barnes, 1959).

An important observation in this context is that l-labelled IgG can be transported by FcRn, but that after transport this fraction of IgG is specifically metabolized, while unlabelled IgG remains active (Hemmings, 1956). This might have implications for the concept of testing heterologous (e.g., human) antibodies in animals.

Mice and Rats

The timing of IgG transfer in rats was described by Halliday (1955a). Rat fetuses immunized with Salmonella pullorum antigen during gestation developed from gestation day 17 small but significant amounts of antibodies in their sera (~1% of maternal concentration). In Figure 1, the data from rats, guinea pigs, rabbits, nonhuman primates, and humans are presented. At birth, the concentration had increased by 8 times and by another factor of 8 observed for several days after birth. By day 5, most suckling pups had antibody titers equal to those of their mothers and remained at this value until day 20 of lactation. This finding is confirmed by the work of Zhang et al. (1988), who immunized rats with hemorrhagic fever with renal syndrome virus (before mating) and found that developed antibodies were transferred to the pups both pre- and postnatally, reaching peak concentrations by week 2 of lactation and thereafter declining gradually until disappearance at week 8.

While in humans the majority of the maternal IgG is transferred to the fetus in utero during pregnancy, the IgG in rats, conversely, is transmitted mainly postnatally. Most of the transmission occurs after birth, continuing throughout the greater part of lactation (Halliday, 1955b). This lactational transfer will be described at the end of this section.

No reports were identified in which the maternofetal transfer of mouse IgG has been studied. Mphis and Gitlin (1970) compared the maternofetal transfer in mice of human IgG with transfer of labeled human albumin. Transfer has been shown to start on day 11–12 of pregnancy as from day 12 a sharp increase in IgG is observed over labeled albumin that is transferred passively. As this regards heterologous transfer, the data have not been included in Figure 1.

The FcRn receptor, mentioned earlier, has also been detected in the fetal yolk sac (Saji et al., 1999). The primary source of exchange and early immunoglobulin transport between the embryo and mother during early gestation in rats and mice is the inverted yolk sac, considered as the first functioning placenta before the chorioallantoic placenta circulation is established (Bekman et al., 1990). The uterine secretion is taken up into the vacuoles of the yolk sac endoderm. These vacuoles transport intact antibodies across the membrane and release it into the fetal circulation, at the same time digesting the rest of the proteins acquired from uterine secretion. Thus, the non-selective uptake is followed by a selective transport. Fc receptor for IgG is situated on the vacuole membranes. After IgG binding with the receptor, the complex is internalized and transported to the other side of the cell inside clathrin-coated vesicles. Unbound and excess IgG gets digested in the vacuoles similar to the other proteins (Baintner, 2007).

In addition to this way of prenatal transport, rodent fetuses can also acquire IgG via their intestinal epithelium from swallowed amnion liquid. Compared to the amniotic fluid amounts, there are very low concentrations of IgG in the gastro-intestinal tract of the rat fetus in
contrast to the concentrations found in the gut of fetal rabbits. This finding indicates the rapid absorption of antibody by the fetal rat gut (Brambell and Halliday, 1956), which is probably mediated by FcRn receptors as in human (Israel et al., 1993). In summary, two main routes for antibody transfer into the fetus circulation from uterine lumen are identified for rodents:

- directly through the yolk sac splanchnopleure;
- via the exocoel and reaching the amniotic fluid through the amniotic membrane, and swallowed by the fetus and absorbed from the fetal gut.

However, an additional route might exist, as fetuses in which yolk sacs and oral routes of transfer were experimentally rendered nonfunctional still had significant titers of antibodies (Smith and Schechtman, 1962).

One hypothesis is that the IgG transfer takes place via the crypts of Duval, the areas where the endodermal tissue of yolk sac invades the margins of the chorioallantoic placenta, where it probably contacts maternal blood. If antibody gets absorbed by these endoderm cells, it might be transmitted directly into the allantoic vessels in the crypts of Duval (Brambell, 1970).

In the suckling rat, the IgG is absorbed from the maternal milk and this process is mediated by the FcRn receptors, located on the brush border membrane of the enterocytes (Pichler, 2006). The secretion of HCl and pepsinogen by the neonate is on a low level during the suckling period; therefore, the maternal IgG can reach into the small intestine undegraded. The slightly acidic (pH = 6) lumen of the proximal small intestine allows IgG to bind to the receptor. The receptor-IgG complex is believed to be protected from intestinal proteases. The FcRn-IgG complex is thereafter internalized and transcytosed in the clathrin-coated vesicles. At the basolateral side of enterocyte, IgG is released into the slightly alkaline lymph (Leach et al., 1996; Baintner, 2007). The absorption from the gut drops rapidly after day 20 after birth at the same time as weaning of the animals (Halliday, 1955b).

**Rabbits**

As rabbits are commonly used for studying placental exchange in animals, their yolk sac is a well-studied organ in respect to prenatal antibody transfer (for reviews see Brambell, 1970; Faber and Thornburg, 1983).

Three periods can be distinguished in the functional life of a rabbit yolk sac. In the early period (until about GD 8), antibody transfer to the fluid of the primary yolk sac appears mainly through the uterine lumen and through the early bilaminar non-vascularized yolk sac membrane. During the second period (GD 8–13), the inversion process with the morphological changes of the yolk sac occurs and antibody transfer is minimal (Brambell et al., 1949). This finding was confirmed by the experiments of Smith and Schechtman (1962) where human serum proteins, injected intravenously to pregnant rabbits on GD 5–8, were present in fetal serum at GD 13. No transfer occurred to the fetuses at GD 13 when the pregnant females were injected at GD 11. The authors hypothesized that proteins present in foetal serum at GD 13 might have deposited into the yolk sac fluid already before GD 9 and a degrading bilaminar yolk sac wall was acting as a barrier to the passage of proteins from GD 9–13.

The last period (GD15 until term, GD32) is the most thoroughly studied and the majority of IgG transport is found to take place during this period. In this period, the endoderm of the yolk sac is in close contact with the uterine epithelium, allowing the most efficient transport. Kulangara and Schechtman (1962) have shown in their studies that human IgG, administered intravenously to pregnant rabbits, is transmitted to the fetus at GD 19, but the transfer is higher on GD 24. The IgG levels of newborn rabbits were found to approximate those of their mothers.

The antibody transfer across the inverted yolk sac in rabbit is mediated by acid-independent Fc receptors (Meads and Wild, 1994). The mechanism of IgG binding and the transmission of the complex across the endoderm of the yolk sac is similar to that already described with the rodent yolk sac transfer. The main role of the inverted yolk sac placenta in the transport of antibodies is proven by experimental manipulation of yolk sac in several studies. For example, antibodies from rabbit antiserum were found to be transferred to the fetal plasma, but in the case where the yolk sac vascularizing blood vessels were ligated, no antibody transfer to the fetus circulation was seen, although antibodies did accumulate in the amniotic fluid (Kulangara and Schechtman, 1962).

Labeled antibodies have also been found to accumulate in the fetal gut, indicating that even though rabbit fetuses regularly swallow amniotic liquid, no antibody transfer takes place through their intestinal epithelium (Brambell et al., 1948; Kulangara and Schechtman, 1962; Baintner, 2007). The experiments of Brambell et al. (1951) indicate that in rabbit the transfer from the gastrointestinal route compared to the yolk sac route is much less important or even absent.

In rabbits, in contrast to other species discussed here, there is also significant class IgM antibody transport through the placenta (Baintner, 2007). Merad and Wild (1992) demonstrated that uterine fluid surrounding the rabbit conceptus contained endogenous IgG and IgM in the second half of the pregnancy and that IgM was transported to the fetal blood through the yolk sac splanchnopleure.

**Guinea Pigs**

Guinea pigs, in comparison to other rodents, have a very long gestation period (68 days) and, therefore, their pups are remarkably developed when born. The embryonic membranes, although similar to that of other rodents such as rats and mice, are arranged in a different way. The main difference is that the yolk sac splanchnopleur is attached near the margin of the placenta rather than that it invest the allantoic stalk. The sides of the placenta are covered with endoderm, and crypts of Duval are not formed. The placenta itself is of the hemochorial type just as in rats and mice; however, it consists of 1 trophoblastic layer as opposed to 3 layers in rats and mice.

Barnes (1959) has reported that antibodies could not be found in the circulation of fetuses before the 33rd day of gestation.
The transmission of immunity takes place only before birth and it seems that both yolk sac and fetal gut are involved. Barnes (1959) could not find any evidence for a pathway via the gut but experiments by Leissring and Anderson (1961a) showed that the yolk sac is the main route of transmission up to GD 40 and fetal gut after GD50. Antibodies were first detected in fetal circulation at GD 35 and their levels increased when gestation proceeded, reaching half to equivalent of maternal levels for GD 50 and exceeding maternal level with a factor 2, with a small decrease near term (Barnes, 1959) (see Fig. 1).

The transmission of IgG has shown to be reduced, when the yolk sac vessels were ligated (Leissring and Anderson, 1959b).

From previous studies with pepsin-digested antibodies (representing Fab-fragments) in guinea pigs, it is clear that the maternofetal transfer of these fragments is very low to absent (Brambell et al., 1959), whereas the papain-digested fragment III (the fragment representing the Fc part) could be readily transferred (Brambell et al., 1960) as has been confirmed in rabbits (Tsay and Schlamowitz, 1978).

As in rabbits, the maternofetal transfer of heterologous antibodies has been studied and found to be less effective (Al-Najdi, 1965). This might be an important drawback when using guinea pigs for testing the developmental and reproductive toxicity of monoclonal (i.e., human) antibodies.

Non-Human Primates

The most extensively used non-human primates in non-clinical trials concerning immunological products are Old World monkeys including rhesus and cynomolgus macaques, baboons, and, rarely, the chimpanzee. Their immunological system is so similar to the human that many immunological human pharmaceuticals are functional in these species (Golos, 2004).

Chimpanzee is the primate species most closely related to humans, but the ethical dilemmas surrounding their use in laboratory research coupled with their endangered status are the limiting factors for using this species.

The monkey species used preferably in reproductive/developmental toxicity studies is the crab-eating or Cynomolgus monkey (Macaca fascicularis) rather than the Rhesus monkey. The Rhesus monkey is a seasonal breeder, whereas the Cynomolgus monkey has a regular oestrus cycle and has a nearly similar gestation cycle (155 in Cynomolgus vs. 165 days in Rhesus). About 30% of them have discoidal placenta as in humans, the other 70% of Cynomolgus monkeys have bidiscoidal placenta similar to Rhesus monkey (Van Esch et al., 2008). The start of embryonic development and organogenesis of the Cynomolgus monkey is similar to that of the Rhesus monkey (Hendricks and Cukierski, 1967; Coe et al., 1994).

IgG transfer in macaques is via the chorioallantoic placenta, as the yolk sac in these species does not have the same function as in rodents and rabbits. The offspring of Rhesus monkey species are born with 80–100% of adult IgG levels (Coe et al., 1993). The antibody transfer to the fetus mainly takes place during final 50 days of gestation. These authors found in their study on Rhesus monkeys that a large increase in IgG transfer occurs during the last two weeks of pregnancy. Fujimoto et al. (1983) showed in Cynomolgus monkeys the exponential increase in IgG transfer starting from GD84. They also demonstrated that IgG levels of the newborn monkeys remained somewhat lower than the levels in their mother.

In Figure 1, an increase in IgG transfer during pregnancy in the Rhesus and Cynomolgus monkeys (taken together as nonhuman primate data) is compared to the human, rodent (rat and guinea pig), and rabbit IgG fetal/maternal ratio.

OVERVIEW OF REPRODUCTIVE TOXICITY STUDIES WITH MONOCLONAL ANTIBODIES

Selection of Species for DART Studies of Monoclonal Antibodies

Several aspects have to be considered before making a choice for a species in which developmental and reproduction toxicity (DART) studies for monoclonal antibodies as human pharmaceuticals will be carried out.

It was recognized during the last 10–15 years that such studies should be done preferably in a relevant species, which means being responsive to the pharmacological properties of the compounds (Guideline ICH S6, Martin, 2008). So, for monoclonal antibodies one test species that is pharmacologically responsive is sufficient to fulfill this criterion. Also other criteria in species selection are applicable, which will be discussed below.

Based on the Guideline on Reproductive Toxicity (Guideline ICH S5), three different study designs are commonly used, i.e., the Fertility and Early Embryo Developmental Design (FEED) with administration from before mating until implantation, the Embryo-Fetal Developmental study (EFD) with administration from implantation to cleft palate closure, and the Pre (peri)- and Post-Natal Design (PPND) with administration from cleft palate closure until weaning. The principles underpinning these designs are valid irrespective of the species under study, although the precise dosing schedule will be different. It has to be acknowledged that the guideline allows sponsors to use other types of study designs, when justifiable, but this does not occur frequently.

Important considerations in the study are the group size and the number of pups per litter. Availability of historical data is a preferred situation. As antibodies are known to have a high species specificity, an important aspect is the potential induction of immunogenicity, which is more likely to occur in more distantly related animal species.

It is preferable to have all aspects of reproduction toxicity studied in the same species. Performing mating studies in nonhuman primates to fully assess effects on fertility and early embryonic development (FEED) is not practicable because of the low number of animals involved. Weinbauer et al. (2008) described the parameters that can be used to evaluate female and male fertility in a non-human primate model.

Therefore, testing potential influences of human pharmaceuticals on fertility is also important, but for monoclonal antibodies it is agreed that histopathological evidence from repeated-dose toxicity studies, coming from relevant organs, might be sufficient in most cases.

Rats and mice are used extensively in DART studies for common pharmaceuticals due to their short gestation period (see Table 1), short estrus cycle, and low-cost maintenance. The number of fetuses of these species is also an important advantage. However, the use of these
species in safety testing for monoclonal antibodies is hampered both by lack of pharmacologic responsiveness and by immunogenicity (Chapman et al., 2007).

To overcome these problems, companies are developing so-called homologues as surrogates, products against a mouse-specific antigen (e.g., a mouse-specific cytokine) with a similar specificity, and with a mouse-specific backbone, i.e., Fc part, making the mouse a pharmacologically responsive species. With recombinant techniques, it might be possible to use the same variable parts as in the human specific clinical candidate with a mouse backbone. When the intended epitope, for instance a membrane protein, is not present in the mouse, then development of a transgenic mouse incorporating the appropriate human gene might be an additional option. This type of approach has been used several times already for products that reached the market (see Studies Performed for Marketed Monoclonal Antibodies).

However, even if the adult rodent model is pharmacologically responsive and exposures are adequate, the maternofetal IgG transferred prenatally is low, and the study design should be extended to include the full lactation period in order to become a good biologic model for human pregnancy. This is explained in the legend to Figure 1.

The rabbit is one of the most popular animal species used to study reproduction toxicity, and it has also a short duration of gestation (68 days up to maturatation of the pups). Maternofetal transfer via the yolk sac splanchnopleur exerts the same time profile as in human. Also for this species, limitations can be expected from the lack of pharmacological responsiveness and the development of immunogenicity, as with rodents. The use of rabbits for testing fertility is less common, and lack of background data might be an important hurdle in this respect.

Guinea pigs might be seen as a potential model because of the duration of pregnancy (68 days up to maturatation of the pups). Maternofetal transfer via the yolk sac splanchnopleur exerts the same time profile as in human. Also for this species, limitations can be expected from the lack of pharmacological responsiveness and the development of immunogenicity (see Guinea Pigs section). Development of a guinea pig-specific surrogate has not been described thus far. Lack of background data might be an important gap in the applicability of guinea pigs in this respect.

Monkeys have in the chorioallantoic placenta the most similar placental function in an anatomical sense as compared with humans. There is the drawback of the low number of offspring and the ethical/societal issue per sé of using non-human primates. In addition, non-human primates have a high rate of spontaneous abortion (up to 28%) (Hendrie et al., 1996). Furthermore, they have low fertility and a long gestation time (155–165 days). Therefore, in a typical developmental and reproductive toxicity study in non-human primates, the number of animals is in the order of magnitude of 48–64 (12–16 females in a group, with 3 dosage groups) (Martin, 2008; Van Esch et al., 2008; Chapman et al., 2007).

### Studies Performed for Marketed Monoclonal Antibodies

From the European Public Assessment Reports (EPARS) at the EMEA website (www.emea.europa.eu), information has been gathered for 18 mAbs and for 2 fusion proteins registered between 1995 and 2008 within the EU. A brief overview of each compound was made, consisting of antibody subtype, way of production, mechanism of action, type of indications, and administration.
<table>
<thead>
<tr>
<th>Generic/trade name</th>
<th>Description</th>
<th>Therapeutic category</th>
<th>Approval date in EU</th>
<th>Reproduction studies</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abatacept Orencia</td>
<td>Human IgG Fc domain + CTLA-4; anti-CD80 and anti-CD86</td>
<td>Immunological</td>
<td>02/03/00</td>
<td>EFD: mice, rats, rabbit PPND: rats</td>
<td>–</td>
</tr>
<tr>
<td>Adalimumab Humira</td>
<td>Human, IgGlκ, anti-TNFz</td>
<td>Immunological</td>
<td>09/1/03</td>
<td>Combined EFD and PPND: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Alemtuzumab MabCampath Basiliximab Simulect</td>
<td>Humanized, IgGlκ, anti-CD52</td>
<td>Oncological</td>
<td>07/06/01</td>
<td>RDT: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Bevacizumab Avastin</td>
<td>Humanized, IgGlκ, anti-vascular endothelial growth factor</td>
<td>Oncological</td>
<td>01/12/05</td>
<td>EFD (2); rabbit RDT: Cyn</td>
<td>EFD rabbit: dose-dependent decrease in maternal body weight, increase in fetal malformations and late resorptions; RDT Cyn: dose/treatment duration dependent inhibition of ovarian function in females, body weight reduction in males Weight loss and reduced food consumption in high-dose group Dose-dependent increase in abortion rates (not known, if could be associated with treatment)</td>
</tr>
<tr>
<td>Cetuximab Erbitux</td>
<td>Chimeric, IgGlκ, anti-epidermal growth factor receptor</td>
<td>Oncological</td>
<td>06/29/04</td>
<td>RDT: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Daclizumab Zenapax</td>
<td>Humanized, IgGlκ, anti-CD25</td>
<td>Immunological</td>
<td>02/26/99</td>
<td>PPND: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Eculizumab Soliris</td>
<td>Humanized, IgG2k, anti-human C5</td>
<td>Immunological</td>
<td>20/06/07</td>
<td>FEED, EFD, PPND: transgenic mice</td>
<td>–</td>
</tr>
<tr>
<td>Efalizumab Raptiva</td>
<td>Humanized, IgGlκ, anti-CD11a</td>
<td>Immunological</td>
<td>09/20/04</td>
<td>FEED, EFD(2), PPND: mice with homologue muM17</td>
<td>–</td>
</tr>
<tr>
<td>Etanercept Enbrel</td>
<td>Human IgG Fc domain + TNFR2/p75; anti-TNFz</td>
<td>Immunological</td>
<td>05/21/07</td>
<td>RDT: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan Zevalin</td>
<td>Murine, IgGlκ, anti-CD20; radiolabeled (Yttrium 90)</td>
<td>Oncological</td>
<td>01/16/04</td>
<td>FEED, EFD: mice with homologue V1qmuG2a</td>
<td>–</td>
</tr>
<tr>
<td>Infliximab Remicade</td>
<td>Chimeric, IgGlκ, anti-tumor necrosis factor (TNFα)</td>
<td>Immunological</td>
<td>08/13/99</td>
<td>FEED, EFD: mice with homologue V1qmuG2a</td>
<td>Reduction in fertility (not known, whether related with male or female animals)</td>
</tr>
<tr>
<td>Natalizumab Tysabri</td>
<td>Humanized, IgG4k, anti-α4-integrin</td>
<td>Immunological</td>
<td>06/27/06</td>
<td>FEED, EFD(2): Guinea pig EFD, PPND – Cyn</td>
<td>EFD G. pig: reduced pregnancy rates in high-dose group PPND Cyn: increased abortion and stillbirth rates</td>
</tr>
<tr>
<td>Omalizumab Xolair</td>
<td>Humanized, IgGlκ, anti-IgE</td>
<td>Immunological</td>
<td>10/25/05</td>
<td>FEED, EFD, PPND: Cyn</td>
<td>–</td>
</tr>
<tr>
<td>Generic/trade name</td>
<td>Description</td>
<td>Therapeutic category</td>
<td>Approval date in EU</td>
<td>Reproduction studies</td>
<td>Findings</td>
</tr>
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</tr>
<tr>
<td>Palivizumab Synagis</td>
<td>Humanized, IgG1k, anti-respiratory syncytial virus</td>
<td>Anti-infective</td>
<td>08/13/99</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Panitumumab Vestibix</td>
<td>Human, IgG2k, anti-human epidermal growth factor receptor</td>
<td>Oncological</td>
<td>03/12/07</td>
<td>FEED, EFD: Cyn</td>
<td>EFD: increased frequency of abortion/fetal death rates in high-dose group</td>
</tr>
<tr>
<td>Ranibizumab Luncentix</td>
<td>Humanized, IgG1, anti-human vascular endothelial growth factor, Fab</td>
<td>Tissue growth and repair</td>
<td>01/22/07</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rituximab Mabthera</td>
<td>Chimeric, IgG1k, anti-CD20</td>
<td>Oncological</td>
<td>06/02/98</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sulesomab Leukoscan</td>
<td>Murine, Fab, binds to surface granulocyte non-specific cross-reacting antigen present on neutrophils</td>
<td>Diagnostic</td>
<td>02/14/97</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trastuzumab Herceptin</td>
<td>Humanized, IgG1k, anti-HER2</td>
<td>Oncological</td>
<td>08/28/00</td>
<td>FEED, EFD, PPND: Cyn</td>
<td>–</td>
</tr>
</tbody>
</table>

FEED, Fertility and early embryonic development studies; EFD, embryo-fetal development studies; PPND, pre- and post-natal development studies; RDT, repeated dose toxicity studies; Cyn, Cynomolgus monkey; >, no reproduction studies (in column Reproduction studies); », no findings (in column Findings).

### Summary

<table>
<thead>
<tr>
<th>Number of products</th>
<th>Type of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynomolgus monkeys</td>
<td>11</td>
</tr>
<tr>
<td>Mice with homologues</td>
<td>3</td>
</tr>
<tr>
<td>Rabbit + Cyn. monkey</td>
<td>1</td>
</tr>
<tr>
<td>Guinea pig + Cyn. monkey</td>
<td>1</td>
</tr>
<tr>
<td>Mice, rats, rabbits</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>į</sup>fertility in RD: fertility was covered by histopathological parameters, not functional tests

<sup>į</sup>PPND in cynomolgus monkeys in some cases starting at GD120 as a peri-postnatal study.
addition, a list of reproductive toxicity studies performed was created, which includes information about the types of studies, animal species used, dosage and administration regime, and observed effects (Table 3).

Thirteen products were IgG1 based, 2 contained a frame of other IgG subtypes (IgG2 and IgG4), and 4 consisted of antibody fragments (2 products contained IgG Fc part and 2 products Fab’s). Since the vast majority of the therapeutic monoclonal antibodies are IgG1 based, they are expected to be transported across the placenta.

In studies with 7/20 mAb products, Cynomolgus monkeys were used as the only relevant species for toxicity studies due to the species specificity of antibody therapeutics. Three out of 20 cases included studies on mice, using mouse antibody homologues and/or transgenic mice, in order to mimic the clinical situation.

Only on 3 occasions was more than one species (Cynomolgus monkey+guinea pig; Cynomolgus monkey+rat; rat+rabbit+mouse) used, and pharmacological responsiveness of the smaller species has been demonstrated, although sometimes with a lower affinity.

Natalizumab has been shown to have pharmacological responsiveness in the guinea pig. As this species has shown to have mainly prenatal transfer of maternal IgG (see Guinea Pig section), it is a good example of a thoughtful selection of a species for the fertility and embryofetal studies, as is clear from Table 2. Male fertility studies were complicated by unexplained sensitization by natalizumab. An additional study has been done in Cynomolgus monkeys (see Table 3). In the embryofetal study (GD20–GD70), adverse effects were observed as changes in the foetus that included mild anaemia, reduced platelet counts, increased spleen weights, and reduced liver and thymus weights. The mechanism is unexplained thus far (EMEA, EPAR).

Developmental toxicity studies were not conducted with 8/20 mAb-products. In those cases, the manufacturers justified that there was no need to conduct reproductive/developmental toxicity studies because of the intended patient population.

However, in 3/8 of these products (i.e., alemtuzumab, cetuximab, etanercept), effects on reproductive organs were examined during repeated dose toxicity (RDT) studies in Cynomolgus monkeys (see Table 3). Also with bevacizumab, the effect on male reproductive organs was examined separately in a repeated dose toxicity study in Cynomolgus monkeys.

In one case (palivizumab), the complete lack of a relevant animal model (anti-RSV mAb) was a reason for not performing reproductive toxicity studies.

For 8 out of 15 compounds, for which reproductive/developmental toxicity studies (in a broad sense) were performed, no significant maternal, fetal, or neonatal toxicity was observed. For the remaining 7 products, the most common adverse effects on reproduction and development were reduced fetal weight, increased abortion rates, and reduction in fertility, indicating the general toxicity of these compounds. These effects showed a tendency for dose-dependency. For 2 products (daclizumab and natalizumab), there was an increase in abortion rates in the Cynomolgus monkey with an equivocal association with drug exposure.

Antibodies against TNF-α (infliximab, adalimumab) are expected to impair fetal/embryonic development since knocking-out TNF-α in mice has been found to increase embryonic death and structural anomalies compared to wild-type mice (Toder et al., 2003). Torchinsky et al. (2003) indicated that TNF-α itself seems to function as a protective agent to development toxicity caused by cyclophosphamide. However, the reproduction/developmental toxicity studies with adalimumab (combined EFD and PPND in Cynomolgus monkeys) and infliximab (homologue in mice) did not reveal maternal or fetal toxicity (EPARs, Roux et al., 2007). In addition, another TNF-α antagonist, golimumab (still in development), administered to Cynomolgus monkeys throughout pregnancy and lactation did not show any adverse effects on the development and maturation of the immune system of the offspring (Martin et al., 2007).

Bevacizumab is an antibody directed against human vascular endothelial growth factor (VEGF) and its ability to inhibit the angiogenesis of tumor cells is currently being applied in treatment of metastatic colorectal and breast cancer. Important is the EFD study in rabbits with Vascular Endothelial Growth Factor (VEGF) receptor inhibitor bevacizumab, where fetal malformations were detected in the high-dose group. As could be expected from its pharmacological activity and has been shown also in repeated toxicity studies on Cynomolgus monkeys, bevacizumab was found to inhibit the ovarian function. As well as playing an important role in follicle and corpora luteal development, angiogenesis is clearly critical in embryo-fetal development as shown in mice (Carmeliet et al., 1996) and marmosets (Rowe et al., 2002). This might explain the fetal deaths and malformations seen following administration of Bevacizumab to pregnant rabbits. Robinson et al. (2007) mention the possible causative role of bevacizumab in the development of pre-eclampsia-like syndrome, based on the occurrence of hypertension and proteinuria (Stoehlmacher, 2007; Cohen et al., 2007). Antagonism of the VEGF system may cause hypertension in humans (Zhu and Perazella, 2007) and VEGF inhibition (caused by intrauterine hypertension) might cause pulmonary hypertension, as observed in the ovine fetus (Grover et al., 2003). No human experience is present thus far. Based on the knowledge described above that monoclonal antibodies are crossing the placental barrier to a low extent during the period of organogenesis, it is more likely that the effect of bevacizumab is rather indirect, i.e., influencing the growth and function of the placenta.

Where adverse effects have been seen in the Cynomolgus monkeys, so far these have been abortions and still births rather than malformations (with natalizumab inducing changes in organ weight). This spectrum of detected effects indicates that of the pharmacological targets assessed so far, the abortifacient effects may be the most serious, and that these abortifacient effects can occur even if direct fetal exposure to the mAb during early gestation is limited. Hendrie et al. (1996) indicate that abortion rates may vary in periods of years between 9 and 28% whereas Small (1982) described a range of 11–30%.

Therefore, it might be that the abortifacient effects are related to chance findings in certain study populations.

**EXPERIENCE WITH MABS IN HUMAN PREGNANCY**

Once a product has been marketed, the product might be applied occasionally in women of child-bearing
potential, but accrual of human data on pregnancy outcome is a slow and often badly coordinated process. Integrating animal and human data is, however, important in human risk assessment.

Table 4 lists mAb/soluble IgG fragment products for which human experience has been reported thus far. For anti-TNFα mAbs, there is more experience than for the other products mentioned, and in fact for some products there are no published data. The variability in data quantity presumably reflects whether that disease is prevalent in women of reproductive age as well as the length of time post-marketing.

It should be kept in mind that the experience in humans will be influenced by the possible effects of the underlying disease on pregnancy outcome. Crohn’s disease, an active inflammatory bowel disease, is associated with poor pregnancy outcome (significantly increased risk for preterm delivery and low-birth-weight infants) and, therefore, providing safe treatment options during pregnancy is a concern for both patients and physicians.

Anti-TNF-α mAbs: Infliximab, Adalumimab, and Etanercept Infliximab

A large study on an infliximab safety database owned by the manufacturer (Katz et al., 2004) reported on 131 pregnant women with RA and Crohn’s disease (CD) exposed to infliximab (mainly around conception and/or during first trimester of the pregnancy). Pregnancy outcome was known for 96 of them. Live births occurred in 67% (64/96), miscarriages in 15% (14/96), and elective termination in 19% (18/96). These results are similar for general populations of pregnant women and pregnant women with CD not exposed to infliximab in the United States. No increased risk of adverse outcome was detected. Five infants were born with complications: one child was born during WG24 and did not survive, a second child had a complicated neonatal course, a third child was born with Tetralogy of Fallot, and a fourth child with intestinal malrotation. One twin from a set of twins had developed delay and hypothyroidism.

In the case of the premature child, infliximab was administered in combination with azathioprine, metronidazole, and mesalamine early in the first trimester. The mother had active CD during conception that might have contributed to the negative outcome of this pregnancy. The two children with fetal abnormalities (Tetralogy of Fallot and intestinal malrotation) were born about 1 year before conception and during the first trimester of the pregnancy. The mother of the child with intestinal malrotation had received infliximab before conception and continued to receive it during her pregnancy. She also was receiving leflunomide, which is a known teratogen in animals (Katz et al., 2004), but has not produced a teratogenic effect thus far in humans (Brent, 2001).

In a report on 10 women, three premature infants and one with low birth weight were born. Eight women were receiving infliximab throughout their pregnancy, one during the first trimester and one’s treatment was initiated in the last trimester. Most of the patients also received other treatments (purine analogues, steroids, etc.) in addition to infliximab some time during pregnancy. Two cases of neonatal illnesses were reported, a neonatal jaundice in one child and severe respiratory distress in another. The mothers of both children were receiving infliximab as a maintenance dose throughout pregnancy for treatment of Crohn’s disease. The relationship to maternal infliximab use is not known (Mahadevan et al., 2005).

In four case reports with the mothers with Crohn’s disease, pregnancy ended with the live births, two full-term and two pre-term (<WG37; one WG36 and one WG34.5), and the children were healthy at last follow-up. In all cases, administration of infliximab was continued throughout pregnancy (Mahadevan et al., 2005; Tursi, 2006; Rosner et al., 2007).

Adalimumab. The data about adalimumab is limited to a few case reports in which the women had an uncomplicated pregnancy and delivered a healthy infant at term. In two cases, the women were exposed to adalimumab throughout the whole pregnancy and in the third case starting from WG 20. In another case report, the delivered child was immature; however, the mother was reported to have active rheumatoid arthritis, referred to as a possible explanation for this premature delivery. The adalimumab was administered as a single dose during the first trimester (Robinson et al., 2007; Mylonaki et al., 2006; Coburn et al., 2006).

Presumed VACTERL Association. Recently, concerns have been raised about a possible causative role of anti-TNF-α agents use by pregnant women in the development of VACTERL (previously VATER) association type birth defects in infants. VACTERL (V: vertebral defects, A: anal atresia or imperforate anus, T: cardiac abnormalities [most commonly atrial septal defect, ventricular septal defect, and tetralogy of Fallot], R: tracheoesophageal fistula or tracheal atresia/stenosis, E: esophageal atresia, R: radial and/or renal abnormalities, L: limb abnormalities) is a rare association of birth defects occurring in 1.6/10,000 live births (Carter et al., 2006, 2008). In a review of cases derived from the FDA database about adverse events associated with
TNF-antagonists, collected until December 2005, they report a total of 41 children with congenital anomalies (total number of users not given). Infliximab has been used to treat 19/41 of mothers at some point during their pregnancy, 22/41 were on etanercept (dimerized p75 TNF receptor); there were no reports on women taking adalimumab. Twenty-four (59%) of the 41 children had one or more congenital anomalies that are part of VACTERL association, but only one child (with maternal etanercept administration) was diagnosed with VACTERL association. In 24 (59%) cases, there was no other medication (10 mothers on infliximab only).

From the cases with infliximab exposure discussed above, children with tetralogy of Fallot, intestinal malrotation, and severe respiratory distress (study of 10 women) were thought to be characteristic to the anomalies of VACTERL association.

However, the study is difficult to interpret as a comparison with the prevalence of spontaneous occurrence of VACTERL is lacking, and the association appears to have received too much emphasis, as the strict criteria for application (at least 3 symptoms) has not been used consistently (even only in one case). Aspects such as co-medication and the effects of the disease itself did not get sufficient attention.

Clearly further data are needed to draw conclusions with regard to a causative role of anti-TNFα mAb in these adverse pregnancy outcomes. Based on the general effects of TNF-α deficiency in knock-out mice, the VACTERL association is only weak if this would be related to TNF-α antagonists, whereas on the other hand, the effects of infliximab and adalimumab in mice and monkeys, respectively, are rather weak or even absent.

Very recently, Berthelot et al. (2009) reported 15 exposed pregnancies (infliximab 3; adalimumab 2; etanercept 10). Two resulted in miscarriages and one was electively terminated, whereas the remaining 12 babies were in good condition at birth, without apparent malformation.

The animal data on TNF-α function and pregnancy are difficult to interpret in this regard. Blockade of TNF-α functioning by the antagonists appears to be less damaging than knocking-out the gene (as in the TNF-α/-/- mice) (see Studies Performed for Marketed Monoclonal Antibodies).

Based on the period of IgG transfer across placenta (significant transfer starts from WG 13–18), the exposure of monoclonal antibodies during organogenesis is minimal. The normal role of TNFα in pregnancy and placental function in primates is not fully elucidated so it is not possible to rule out on the basis of low fetal exposure to the mAb during organogenesis that TNF antagonism could not lead to malformative events. Therefore, in order to draw final conclusions, further data collection is needed.

It has been mentioned already that the underlying disease might have a negative impact on pregnancy. From that perspective, it might be that the benefits of infliximab and adalimumab use in achieving response and maintaining remission in mothers with CD outweigh their potential risks (Vesga et al., 2005).

### Anti-ERBB2 mAb: Trastuzumab

Trastuzumab is an antibody directed against the ERBB2 (HER2) receptor, a member of the human epidermal growth factor receptor (EGFR) family, and is currently being applied in breast cancer treatment. The human data consist of seven case reports (see Table 5).

Reported detrimental effects (oligohydramnios) of trastuzumab seem to be related to both timing of exposure and to the length of treatment, indicating that there is an increased risk when administered after the first trimester of pregnancy. The mechanism of toxicity to the fetal kidneys is proposed to be associated with the different structure of EGFR in the fetal renal-tubule epithelial cells (heterodimer of EGFR and ERBB2 in fetus vs. homodimer of EGFR in adults). Thus, trastuzumab will have a damaging effect on the fetal renal function, but it does not affect the kidneys of the adult (Robinson et al., 2007).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Human Case Reports on Trastuzumab During Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treatment of breast cancer with trastuzumab and vinorelbine during the first half of the pregnancy. Anhydramnios occurred, indicating the adverse effect on the fetal kidneys. This effect was reversible and anhydramnios resolved slowly after trastuzumab was discontinued after WG 23. No adverse effects on the newborn were noticed.</td>
</tr>
<tr>
<td>2</td>
<td>No anhydramnios was indicated and a healthy infant was delivered after maternal exposure to trastuzumab before and shortly after conception.</td>
</tr>
<tr>
<td>3</td>
<td>Exposure of pregnant breast cancer patient to trastuzumab combined with vinorelbine administered weekly from WG 27 indicated constant anhydramnios, but no other adverse effects. Child was delivered at WG 34.</td>
</tr>
<tr>
<td>4</td>
<td>Trastuzumab was given together with paclitaxel during the last trimester of the pregnancy. The fetus developed renal insufficiency and severe oligohydramnios and was delivered at WG 32 by caesarean section. Fetal kidney function returned to normal after delivery.</td>
</tr>
<tr>
<td>5</td>
<td>Trastuzumab was administered until WG 24. Child was delivered by caesarean section at WG 37. The infant developed transient respiratory failure. However, this failure resolved after treatment without any other complications.</td>
</tr>
<tr>
<td>6</td>
<td>A woman developed an ectopic, cervico-isthmic pregnancy while she was on trastuzumab treatment for the previous 14 months. The patient chose to undergo voluntary abortion.</td>
</tr>
<tr>
<td>7</td>
<td>Trastuzumab was administered until WG 23. Maternal therapy was stopped when oligohydramnios was noted. Premature infant was born at 27 weeks. Girl died 4 months after birth because of problems related to prematurity.</td>
</tr>
</tbody>
</table>
Reproduction/developmental toxicity studies of trastuzumab, conducted on Cynomolgus monkeys, did not indicate decreased fertility or fetal harm, but the design of the two studies did not cover a main part of pregnancy, the EFD study had an exposure from GD20–50 as conducted usually, and the PPND study started only at day 120 and lasted up to natural birth (around day 150). (EPAR and SPC, EMEA). It is, therefore, not clear whether the renal effects of trastuzumab on the fetus are human specific or were simply missed in the Cynomolgus monkeys because of the administration period.

Anti-CD20 mAb: Rituximab

Rituximab is a monoclonal antibody that targets CD20 antigen on B-lymphocytes and is used in treatment of non-Hodgkin lymphoma (NHL) both in combination with chemotherapy or as monotherapy.

Taking into consideration the mechanism of action of rituximab, there is a potential risk of B-cell depletion in the fetus, and indeed only transient lymphocytopenia has been reported in one case, but no further adverse effects on the child’s immune response have been reported (Kimby et al., 2004). There are 3 other cases reviewed (Robinson et al., 2007) with rituximab exposure in pregnant women, and in all of them healthy infants were delivered. Rituximab was administered in one case during the first trimester (Ojeda-Urube et al., 2006), in one case during WG 15–25 (Decker et al., 2006), and in another case from WG 21 until term (Herold et al., 2001).

According to the EPARs, no reproductive/developmental toxicity studies in animals have been performed with this compound, because due to the intended indication the product was not expected to be given during pregnancy. A direct comparison between animal and human data is, therefore, not possible.

DISCUSSION AND CONCLUSIONS

As monoclonal antibodies are increasingly used for treatment of chronic diseases, which occur also in women in their fertile period, there is increased likelihood of both inadvertent and pre-planned pregnancy exposure. The goal of this present overview is to present information that will assist in designing future animal developmental toxicity studies.

The comparison of placental structure and maternofetal transfer of maternal antibodies in different species reveals various important facts.

1. In species used for DART studies (rats, guinea pigs, rabbits, monkeys), the transfer of IgG to the fetus is minimal in the first part of gestation (Fig. 1). In rats, there is access via the yolk sac and some transfer via the amniotic fluid and swallowing by the fetus. In guinea pigs and rabbits, the maternofetal transfer takes place mainly via the yolk sac splanchnopleure and not via the fetal gut. In non-human primates, the maternofetal transfer is similar to the situation in humans. There is general agreement that the titers achieved during the first half of gestation are very low in comparison to maternal concentrations.

2. In the above species, the transport using the FcRn receptor increases during the second half of gestation, but the level of fetal IgG achieved by birth varies considerably with species: In rats and mice, it remains low until birth but Old world monkey fetuses may attain the maternal concentration level. Rabbits and guinea pigs also have considerable pre-natal transfer of maternal antibodies.

3. For rats and mice, postnatal exposure to maternal IgG is quantitatively most important (Halliday, 1955b). IgG is secreted in milk of rats and mice and contributes to the immunological protection in the pups until weaning. In contrast, the amount of lactational transfer of IgG in guinea pigs, rabbits, non-human primates, and humans is believed to be very low or absent, where most of IgG secretion is in the first day after birth (Palmeira et al., 2009). Contribution of IgA is more important in protecting mucosal areas in these species (Sadeharju et al., 2007). In line with these observations, infliximab excretion in breast milk is found to be minimal (Stengel and Arnold, 2008; Vasiliaskas et al., 2006).

The preclinical studies on the mAbs that have received marketing authorization in the European Union thus far have mainly used Cynomolgus monkeys (10 out 15 products). However, some products have been assessed in rabbits, guinea pigs, and mice (in the latter case using homologues). For some compounds, adverse effects on pregnancy outcome have been observed. These have included abortions in Cynomolgus studies (with natalizumab) and embryofetal mortality with or without malformations in species such as rabbit (with bevacizumab). These studies reveal that for certain pharmacologies (e.g., anti-TNF-α, anti-VEGF), which presumably interfere with early development and placental function, it might not be necessary to have considerable fetal exposure during organogenesis to produce an adverse pregnancy outcome such as fetal death. Bevacizumab might be a good example where the pharmacological activity inhibiting angiogenesis has interfered both with fetal morphogenesis and fetal viability, the latter presumably through effects on placental development and function. Provided that the rabbit exerts a relevant pharmacodynamic activity for the monoclonal antibody at hand, this species might be a good model for developmental toxicity studies.

In addition, the guinea pig is a species that, unlike other rodents such as rats and mice, has considerable prenatal IgG transfer, which could model humans, and is applicable if proven pharmacologically relevant. In both rabbit and guinea pig, it would be important to understand the influence of immunogenicity on the ability to maintain exposure throughout gestation, not just for the relatively short duration of organogenesis.

Human data are scarce. For TNF-α-antagonists, there is more experience than with other classes, probably because of their broader use in patient populations where pregnancies can be expected to occur. The causal relationship proposed for the VACTERL syndrome is unproven and has limited biological plausibility. In fact, the combination of vertebrate anomalies (to be expected in the 1st trimester) with other functional effects (expected later in pregnancy) is rather unexpected.

However, for other mAbs with human pregnancy data indicating potential for effects on the fetus, the adverse outcomes are biologically plausible based on the known

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effects previously associated with the pharmacology, e.g., EGFR inhibition and renal effects, CD20 antagonism and lymphocyte depletion, and VEGF antagonism and hypertension/pre-eclampsia. For natalizumab, no human experience is available.

Given the low to very low exposure to maternal antibodies during organogenesis, it would seem unlikely that mAbs could have a significant direct impact on development of the embryo. However, this review reveals mAbs can cause adverse effects through exposure in early gestation although the mechanism by which this happens is not always evident. The long half life of mAb in human subjects also means that deliberate or inadvertent exposure of women in the first trimester could give sufficient longevity of exposure into the period when active transport of maternal IgG into the fetus increases.

This means that despite the expected low materno-fetal transfer during the period of organogenesis in animal EFD studies, it is still important to use study designs that can detect hazards during the early embryonic period. However, an EFD study alone using such a short window of administration is not reasonably valid to assess safety for a human pregnancy. It will be important to have animal studies that adequately model the potential for effects in the latter half of human gestation when fetal exposure can be considerable. Although it may be possible to supplement information from a traditional-style EFD study with a second pre- and post-natal study (where dosing begins at the end of organogenesis), where a non-human primate is the chosen species, it is possible to combine the two study designs into a single pre- and post-natal study design where the mother is dosed throughout gestation and the functional and morphologic consequences are assessed in the primate infants. This uses fewer monkeys than running two individual studies and minimizes the risk of failing to detect developmental toxicity.

There is ethical pressure to avoid the use of non-human primates and to give preference to testing the clinical product in other species (including transgenic mice containing the human antigen) or to test a mouse-compatible homologue rather than the clinical candidate. The great differences in materno-fetal transfer and the limited concentrations that enter the fetus before birth seriously compromise the interpretation of rat and mouse studies regarding their relevance to humans. It is at least important to include the full suckling period in the study design (as in a PPND study), when there is a need to use this approach (e.g., due to the absence of another pharmaco- logically responsive species).

A negative outcome does not assure that a product would be safe in the second and third trimester of human pregnancy where prenatal exposure of the human fetus would be considerable. Conversely, if adverse effects on mouse neonatal survival and growth were detected in a PPND rodent study, inevitably further work in a species with a materno-fetal transfer pattern more similar to man would be required to understand the relevance to human pregnancy.

Guinea pigs and rabbits are potential candidates as “alternatives” to the use of non-human primates as the materno-fetal transfer in the last part of gestation is at a similar level in humans. Especially, guinea pigs deserve a good evaluation in this regard although an important issue might be the lack of historical control data for this species. Points to consider (in addition to pharmaco- logical responsiveness) is the impact of heterology (affinity to the FcRn in the yolk sac compared to the homologue IgG) and the general aspect of immunogenicity of the human monoclonal antibody.

Investigators are encouraged to consider these species differences when choosing species and designing packages of studies to assess the developmental toxicity of mAb.

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REFERENCES


Brent RL. 2001. Teratogen update: reproductive risks of Leflunomide (Arava®), a pyrimidine synthesis inhibitor: counseling women taking leflunomide before or during pregnancy and men taking
lufenonide who are contemplating fathering a child. Teratology 63:106–112.


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PLACENTAL TRANSFER OF MONOCLONAL ANTIBODIES


Pediatric Allergy Immunol. DOI: 10.1111/j.1399-3038.2008.00828.x


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