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Review

The use and misuse of animal analog models of human pregnancy disorders

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ABSTRACT

It has been suggested that the differences between placentation in humans and rodents, such as mice, are sufficient to render human pregnancy unique and to justify ignoring data generated using mice. Detailed examination of the placenta–decidua interaction and decidual NK cell composition in humans, and mice, show that the principles are the same. Indeed, the rat placenta is useful in showing an intermediary arrangement between humans and mice. This is consistent with the thesis of Darwin that structures of older species evolve with development of new species to provide a survival advantage. Molecular details may differ between species, but also between individuals given gene polymorphisms. Human data on interaction of HLA-C2 with NK cell KIR receptors has been used to suggest that human pregnancy problems such as recurrent miscarriage, fetal growth retardation, and pre-eclampsia are due to lack of activation of true uterine NK cell (TuNK) functions that promote trophoblast cell growth and invasion which prevents such problems. But when TuNKs bear certain KIR phenotypes, pathology results. It is shown that such mechanisms could only be pertinent in less than one-third of recurrent miscarriage patients. Activated blood-type NK cells that enter the uterus (BuNKs) remain the major effector of pregnancy loss in humans, and this is consistent with data from the mouse. The importance of activated BuNKs in pre-eclampsia and fetal growth retardation merits further investigation as pre-eclampsia and fetal growth restriction are also manifest in the CBAxDBA/2 mouse model where activated NK cells are the initiator of abortions.

1. Introduction

Embryo implantation failure, occult pregnancy loss, recurrent miscarriage, pre-eclampsia, abruptio placenta, fetal growth restriction, and premature parturition are significant and frequent disorders of normal human reproduction. The goal is to elucidate the underlying mechanism(s) of each at the tissue cellular and molecular level, with the intent of diagnosing the problem and treating it successfully. Since it is usually not possible to obtain tissue from pregnant women in a manner that allows a study of problems as they develop, investigators have turned to animal analog models. In an outbred mating typical of humans, the father is histoincompatible with the mother and the embryo, fetus, and placenta express 'foreign' molecules that can be recognized by maternal immune cells. It is logical that immunological mechanisms

Abbreviations: MBL, mannan binding lectin; KIR, killer immunoglobulin-like receptors; RM, recurrent miscarriage; PE, pre-eclampsia; FGR, fetal growth retardation.

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have been proposed to be potential causes of these disorders. The inbred laboratory mouse has been the most studied, as it has a hemochorial placenta, a short gestation period, has defined genetics allowing reproducible experiment conditions, unlike humans the frequency of karyotype-abnormal embryos is low, and the immune system is well characterized and similar to humans (Clark et al., 1999; Clark, 2008). Further, there are defined models such as the CBAxDBA/2 mating system where implantation failure, recurrent miscarriages, and pre-eclampsia may occur (Clark et al., 1999; Clark, 2008; Ahmed et al., 2010).

Moffett and Loke (2006) consider the structure of the human placenta to differ sufficiently from that of rodents such as mice, so data from mice is not necessarily relevant to the human situation. In humans, there is deep invasion of the decidua by trophoblasts, and the metabolic exchange area is villous. In the mouse, implantation begins on the anti-mesometrial side of the uterus, and the placenta is then established on the mesometrial side and is more superficial. The metabolic exchange area is labyrinthine. Possibly due to direct extrapolation of findings in mice to humans without checking validity, mouse data has been discounted and ignored by some human placenta immunologists. Perhaps the best example of inappropriate extrapolation has been the belief that "blocking antibodies" may explain the success of a semiallogeneic embryo by promoting tolerance, or non-rejection, of the embryo by maternal T cells (Beer, 1988). "Blocking antibodies" are detected by inhibition of maternal anti-paternal reactions in a mixed leukocyte culture in vitro. In the CBA strain of mice, antibodies were shown to prolong survival of allografts by a process called "immunological enhancement" rephrased as "immunological facilitation" (Voisin, 1971). Such antibodies could block MLR reactions. However, the immunological enhancement of allograft survival is limited to CBA strain mice, tolerance is MHC-restricted and hence a T cell phenomenon as antibodies are not MHC-restricted, and tolerance may involve antigens not usually recognized by antibodies. Further, in unexplained recurrent miscarriages in women where miscarriages were prevented by paternal mononuclear cell immunization, blocking antibodies were not required for prevention of abortions within 80 days after administration of paternal cells, but anti-paternal antibody did correlate with prolonged protection after 80 days (Mowbray and Underwood, 1991). Immunological enhancement of the survival of paternal cells expressing tolerance signaling molecules within the female might be antibody mediated, although that has not been formally demonstrated (Clark, 2008).

The rejection of animal data as relevant to humans has been as controversial as the June 1860 debate over Darwin's theory of evolution. The similarity of humans to other species arose due to development and adaptation of common mechanisms. At the 1860 Oxford debate, science won out over authoritarian religious dogma that viewed man as a unique creation different from animals. Science examines carefully the similarities and differences between two species to find out what can be learned. In the case of reproductive problems, studies of the mouse have indeed provided useful ideas about what to look for in human pregnancy. For example, NK cells, TGF-β_2-producing suppressor cells in deciduas, and Treg cells were all shown to be relevant to mouse pregnancy outcome and then validated by testing tissues from successfully pregnant and spontaneously aborting women (reviewed in Clark, 2008). Further, the possible limited applicability/relevance of a single inbred mouse model to a syndrome such as recurrent miscarriage, a syndrome with many different causes, is recognized, as is the different environmental conditions that pertain for mouse versus human existence. Recently the laboratory rat has been developed as a model, in part because it has deep placental implantation, like humans. Like the mouse, initial implantation is on the anti-mesometrial side of the uterus, so the rat has both mouse and human structural features.

2. Structural commonalities in human, rat, and mouse placentation

Differences in placental structure have been mentioned above, There are three categories of similarities which are important (Fig. 1):

1) The trophoblast at the interface with maternal uterine decidua invades the deciduas, and the extent to which this occurs is human > rat > mouse, but it does occur in the mouse (Redline and Lu, 1989). In humans, trophoblasts invade the walls of blood vessels feeding the placental, and this invasion can occur from the decidual side (as best seen in humans) and from the endovascular side. Endovascular trophoblast plugs reduce blood flow, and this is seen in humans, rats, and mice (Adamson et al., 2002; Sebire et al., 2002; Kaufmann et al., 2003; Caluwaerts et al., 2005; Croy et al., 2012; Agostinis et al., 2012). Failure of endovascular invasion and plug formation can lead to hyperperfusion and oxidative damage to the placenta, and inflammation and thrombosis can result (Jauniaux et al., 2000; Safronova et al., 2003; Hempstock et al., 2003). The pathways include up-regulation of FGL2 (fibrinogen-like protein) and complement activation (Knackstedt et al., 2001, 2003; Clark, 2008). It follows that mechanisms which amplify this process increase the likelihood of oxidative damage. If further follows that non-oxidative activation of these pathways could have the same outcome for a pregnancy.
2) Paternal MHC antigens are expressed by trophoblasts at the interface with maternal deciduas. In humans the Class 1a MHC HLA-C is expressed in extravillous trophoblasts, in the rat RTA-1A is expressed, and in the mouse H-2K is expressed in trophoblast giant cells (Kanbour et al., 1987; Saito et al., 1990; Moffett and Loke, 2006; Madeja et al., 2011). H-2K may be expressed by mouse spongiotrophoblasts (Wegmann, 1981). It is less certain if endovascular trophoblasts also express paternal Class 1 MHC because those studies have not been done. In humans, non-classical Class 1b MHC HLA-G and E are expressed, in the rat RTA-Pa, but in the mouse no non-Classical MHC antigen is expressed (Kanbour et al., 1987; Saito et al., 1990; Moffett and Loke, 2006). Trophoblast MHC does not effectively present antigen to T cell receptor for antigen, although interaction with