
Perspectives in Pharmacology

Characteristics of the Fetal/Maternal Interface with Potential Usefulness in the Development of Future Immunological and Pharmacological Strategies

KENNETH L. AUDUS, MICHAEL J. SOARES, and JOAN S. HUNT

Department of Pharmaceutical Chemistry (K.L.A.), University of Kansas, Lawrence, Kansas; Department of Anatomy and Cell Biology, Department of Pathology, and Laboratory Medicine (J.S.H.), University of Kansas School of Medicine, Kansas City, Kansas; Department of Molecular and Integrative Physiology (M.J.S.), University of Kansas School of Medicine, Kansas City, Kansas

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ABSTRACT

A study of the fundamental biology of the maternal-fetal interface reveals the complex interactions among multiple cell types and regulatory factors necessary to support a successful pregnancy. Cells of decidua and trophoblast lineages play central roles in creating the maternal-fetal interface and are sources of regulatory factors that can determine the quality and success of pregnancy. The regulatory factors considered here are major

placental histocompatibility complex proteins, pregnancy-specific regulatory factors for uterine inflammatory cells, and hormone-controlled placental multidrug-resistant transport systems. Potential targets are discussed and presented as areas where researchers may identify novel pharmacological and immunological strategies that eventually will extend to the clinic to improve the quality and success of pregnancy.

The milieu comprising the complex maternal-fetal interface is normally a precisely choreographed interplay among multiple cell types and regulatory factors that results in the immunologically safe and nurturing surroundings required to support growth and development of the embryo and fetus. Generally, two cell lineages, decidua and trophoblast, are involved in both secretion of and responses to several regulatory factors that modify the maternal-fetal interface surroundings to create the necessary conditions and anatomical structures to protect the mother and the fetus. The regulatory factors include chemokines, cytokines, growth factors, antigens, and hormones. On occasion, cell secretions of regulatory factors or responsiveness to these regulatory factors

are disrupted with the ultimate consequences being pregnancy termination, or intrauterine growth retardation, or potentially maternal compromise. As a consequence, characterization of the fundamental biology of the maternal-fetal interface should expose new targets for therapeutic interventions appropriate to protect mother and fetus.

This perspective considers the roles of decidual and trophoblast cells as sources of major placental histocompatibility complex proteins and their influence on maternal inflammatory and immune cell responses, pregnancy-specific regulatory factors for uterine inflammatory cells, and hormone-regulated placental multidrug-resistant transport systems. Although representing three distinct areas, each represents a major player as a protective mechanism for the mother and the fetus. It is clear, for example, that human leukocyte antigens (HLAs) and pregnancy-specific regulatory factors enable the maternal immune system to change and cope with pregnancy and protect the fetal-maternal interface from immune disorders. Based on an understanding of these functions, interventions might someday be developed that enable mothers prone to immune disorders to avoid conditions re-

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ABBREVIATIONS: HLA, human leukocyte antigen; MHC, major histocompatibility complex; NK, natural killer; β 2m, β 2-microglobulin; PRL, prolactin; CG, chorionic gonadotropin; PL, placental lactogen; IFN, interferon; MDR, multidrug-resistant gene product; Pgp, P-glycoprotein; LRP, lung resistance protein; BCRP/MXR/ABCP breast cancer-resistant protein; MRP, multidrug resistance-associated protein.

sulting in spontaneous abortions. Similarly, the emerging knowledge of multidrug resistance mechanisms might eventually be exploited to prevent drug and chemical exposure risks to the fetus. Our discussion of these topics is intended to reveal current knowledge of specific immunological and pharmacological mechanisms and stimulate thoughts of new strategies for developing therapies directed at improving the quality and success of pregnancy.

Major Histocompatibility Complex Proteins and Their Influences on Maternal Inflammatory and Immune Responses

Immunologists were just beginning to understand the dimensions of transplantation and graft rejection in the middle of the twentieth century. During this time, Medawar (1953) proposed the first set of potential explanations for the surprising willingness of mothers to tolerate genetically different tissues during pregnancy. His explanations included anatomical separation between the mother and fetus, antigenic immaturity of the fetus and immunological tolerance in the mother. Although the situation is considerably more complex than originally envisioned, it is now known that essentially all of the explanations proposed by Medawar have an element of truth. Of these, one of the most novel and probably most critical is modulation of expression of the major histocompatibility complex (MHC)-derived cell surface structures known, in humans, as the HLAs.

Trophoblast Cells. Protection for the embryo against the maternal immune system components dedicated to ridding the host of foreign objects rests with the trophoblast layer, and it is this cell type where modulation of HLA gene expression takes place. The inner cell mass from which the embryo proper develops is secluded beneath multiple layers of trophoblastic cells throughout pregnancy. These cells are derived from the external trophoblast layer of the blastocyst. Precursor trophoblast cells choose one or another of three pathways. They may remain quiescent in the villi as a pool of cells for future needs (villous cytotrophoblast cells). Alternatively, they may proliferate and migrate into the decidua (extravillous cytotrophoblast cells). Later in pregnancy these extravillous cytotrophoblast cells form the chorion membrane. In a final option, precursor cytotrophoblast cells may merge to form the syncytialized single cell layer of the placenta termed the syncytiotrophoblast.

These three subpopulations are exposed differently to maternal elements. The villous cytotrophoblast cells are entirely secluded from maternal elements with the exception of any molecules that might be transported across the placenta by the syncytiotrophoblast. By contrast, the extravillous trophoblast cells are continuously exposed to maternal tissues. In early pregnancy, extravillous trophoblast cells are exposed to maternal blood during formation of columns and then are exposed to maternal tissues as they migrate through the decidua (these cells are termed interstitial trophoblast). The early decidua contains significant numbers of natural killer (NK) cells as well as macrophages and possibly also γ/δ T cells. Many trophoblasts ultimately reach the maternal spiral arteries and replace the endothelial cells. These "endo-vascular trophoblasts" are again exposed to maternal blood. Later in pregnancy, extravillous trophoblast cells now forming the chorion membrane are exposed to maternal hemato-

poietic cells, particularly maternal macrophages, which often infiltrate this cell layer. Last, both early and late in pregnancy, the syncytiotrophoblast is continually exposed to maternal blood leukocytes.

HLA Genes and Antigens. MHC complexes found on chromosome 6 in humans and chromosome 17 in mice contain genes encoding two types of transplantation antigens, class I and class II (Geraghty, 1993; Le Bouteiller, 1996). The human MHC complex contains 17 to 20 HLA class I genes, many of which are pseudogenes and gene fragments. Those that are expressed fall into two categories, class Ia and class Ib. Class Ia genes are highly polymorphic and are present as cell surface glycoproteins on essentially all types of cells, the exceptions being gametes and trophoblast. By contrast, class Ib genes encode antigens with limited polymorphism and are frequently restricted in their tissue distribution. Examples of class Ia antigens are HLA-A, -B, and -C; examples of class Ib antigens are HLA-E, -F, and -G. Heavy chain amino acid sequences determine the class designation; nearly all types of HLA class I heavy chains associate with a 12-kDa light chain called β 2-microglobulin (β 2m). The single exception is the class Ib splice variant, HLA-G2. Class II HLA-D antigens are also highly polymorphic antigens, but these are mainly expressed on cells of the immune system.

HLA Class I in Human Placentas. In the human placental bed, extravillous trophoblast cells migrating into the decidua, which later become the chorion membrane, express a unique pattern of class I HLA, with HLA-G, HLA-E, and HLA-C predominating (Hunt and Orr, 1992; Le Bouteiller and Mallet, 1997). In contrast to the migrating extravillous cells, syncytiotrophoblast forming the outermost layer of the placental floating villi, which is exposed to maternal blood, seems to express no membrane-bound HLA class I antigens (reviewed by Hunt and Orr, 1992; Le Bouteiller and Mallet, 1997). In term placentas, this is clearly due to a lack of HLA class I mRNA (Hunt et al., 1988). By contrast, syncytiotrophoblast in the first trimester of pregnancy contains HLA class I message (Hunt et al., 1990) although none seems to encode heavy chains that associate with β 2m as none are detectable with a mouse monoclonal antibody W6/32, which requires β 2m in the binding site.

HLA-G. HLA-G appears to be the predominant HLA class I antigen in/on trophoblast and developmentally regulated with high expression early in pregnancy and lower expression near term. The gene encodes differentially spliced mRNAs that yield several isoforms of the antigen (Ishitani and Geraghty, 1992). Importantly, some isoforms have transmembrane and cytoplasmic domains whereas others do not, being generated from transcripts where a stop codon in intron 4 prevents translation of transmembrane and cytoplasmic domain sequences. In some isoforms, membrane-bound HLA-G2 and soluble HLA-G2, one of the domains required for binding of β 2m is spliced out. The amino acid sequences of membrane bound HLA-G1 predict a traditional peptide binding cleft and association with the light chain, β 2-microglobulin (Fig. 1), whereas the amino acid sequences of HLA-G2 predict a homodimeric heavy chain molecule that would be likely to form a class II-like peptide binding cleft (Fig. 1). Studies on recombinant soluble HLA-G1 and HLA-G2 generated in our laboratory in eukaryotic cells (P. Morales and J. S. Hunt, unreported data) will be examined by X-ray crystallography in the near future. These experiments are

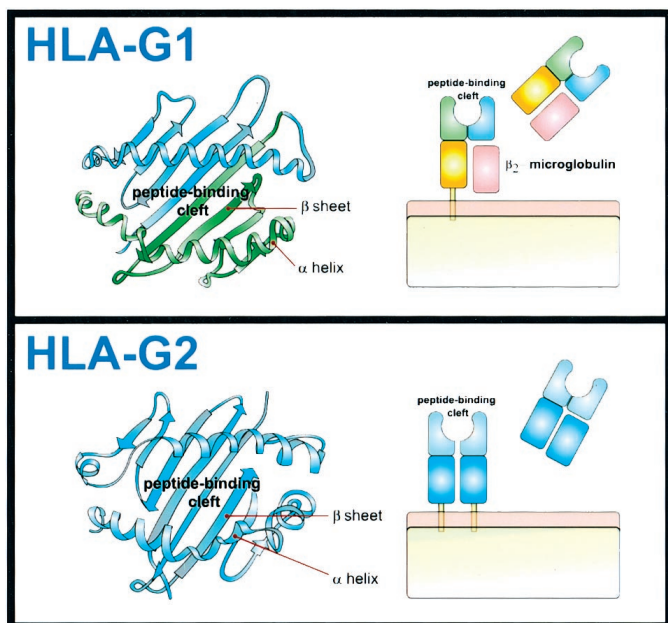


Fig. 1. Schematic representations of HLA-G1 (top panel) and HLA-G2 (bottom panel). Transcripts encoding both membrane-bound and soluble HLA-G1 and HLA-G2 have been reported in human placentas, and the proteins are also present. On the right, the HLA-G1 protein is shown in association with the light chain, β_2 -microglobulin and the HLA-G2 protein is shown as a homodimer. On the left, ribbon drawings demonstrate the peptide binding clefts. In HLA-G1, the $\alpha 1$ and $\alpha 2$ regions of the heavy chain are believed to contribute to the cleft, and in HLA-G2, the heavy chains are believed to form homodimers that constitute the cleft.

expected to resolve questions of secondary structure and whether a peptide binding cleft is formed.

In addition to having multiple splice variants, the HLA-G gene encodes an antigen with cytoplasmic tail that is short in comparison with other HLA class Ia and Ib antigens. Whether this tail can transduce signals into the cell remains unknown. Other class I molecules are effective signal transduction elements. Regulation of this gene is different from other HLA class I genes, which are dramatically enhanced in the presence of interferons. A 16-base pair portion of the enhancer A/interferon consensus sequence found in the promoter region of other HLA class I genes is not present in HLA-G and the gamma-activating sequence (GAS element) is nonfunctional.

The HLA-G1 membrane form is predominant in early placentas but is not essential to pregnancy, as reported in a recent study where a homozygous deletion preventing expression of the HLA-G1 isoform was identified in a healthy, fertile woman with a viable placenta (Ober et al., 1998). In all probability, the HLA-G2 isoform or other, smaller, isoforms may substitute when HLA-G1 is missing.

HLA Class II in Trophoblast Cells. None of the subpopulations of trophoblast cells expresses HLA class II antigens *in vivo*, which may be due to the trophoblast-specific repressor of gene expression identified by Murphy and Tomasi (1998).

Functions of HLA Class I Antigens in Pregnancy. The overall pattern in two subpopulations of trophoblast, syncytiotrophoblast and villus cytotrophoblast cells, is of strict control over the expression of genes that could encode potentially dangerous, paternally derived foreign MHC (HLA-A, -B, -D). As a consequence, the expanded populations of ma-

ternal Th cells required for generation of maternal-antifetal cytotoxic T lymphocytes from precursor cells are missing.

Yet, the fact that some trophoblast subpopulations express selected class I antigens requires explanation. Interestingly, the HLA-G and -E antigens, which have few alleles in comparison with HLA-A and -B, appear to interact with uterine NK cell and possibly also uterine macrophage inhibitory receptors, which include CD94/NGK2A, ILT2 and ILT4. Recent studies in our laboratory have shown that decidual macrophages express the ILT receptors whereas trophoblast cells do not (M. G. Petroff and J. S. Hunt, unpublished results). Thus, placental HLA-G appears to be directed to the mother rather than the fetus.

Class I antigens on trophoblast also interact with TcR on CD8+ cells (Sanders et al., 1991). Based on interactions in other contexts, the consequences probably include activation of killer inhibitory pathways in NK cells and macrophages and possibly death of CD8+ T cells due to activation of the Fas/Fas ligand programmed cell death pathway by soluble HLA class I antigens (Zavazava, 1998). A recent study suggests that soluble HLA-G1 molecules may be able to promote this pathway (Fournel et al., 2000), thus preventing toxicity by HLA-G-programmed T cells.

In summary, the passive and active mechanisms regulating expression of class I molecules undoubtedly provide major protection against maternal immune cells programmed to attack cells expressing foreign (paternal) HLA class I. Concurrently, these antigens may also provide a means for presenting peptides from infectious microorganisms to maintain host defense (Le Bouteiller and Solier, 2001).

Signaling Mechanisms As Modulators of Maternal Uterine Immune/Inflammatory Cells

Semiallogeneic tissue transplantation in most sites of the body results in a carefully orchestrated sequence of events, which under typical circumstances culminates in the rejection and subsequent elimination of the transplanted tissue. The recognition, destruction of foreign cells, and subsequent tissue repair lie with populations of highly mobile cells of the hematopoietic lineage (Croy et al., 1998). The proximal signal initiating the tissue rejection sequelae is the genetic disparity of the transplanted tissue. One of the most prominent exceptions to this transplantation process occurs during implantation of semiallogeneic or allogeneic embryos into the female reproductive tract (Medawar, 1953). Immune and inflammatory cells poised to respond to the alien challenge within the uterus are restructured. For some cell types their entry into the embryo implantation site is attenuated, whereas for other cell types their presence appears to be embraced and actually facilitated (Croy et al., 1998). It is apparent that during pregnancy, the behavior of these cells, which permit us to combat infections and rectify injuries, is exquisitely regulated. The regulators, although poorly understood, are likely soluble molecules that could be classified as chemokines, cytokines, growth factors, or even hormones. Their source has long been hypothesized to be specialized cells present at the maternal-fetal interface.

Sources of Pregnancy-Specific Modulators

Two important cell lineages constituting the maternal-fetal interface are unique to pregnancy (Enders and Welsh,

1993). One is of maternal origin, the decidua and the other is of extraembryonic origin, the trophoblast. Both cell types are likely sources of pregnancy-specific modulators of maternal uterine immune/inflammatory cells.

Decidua. Decidual cells are modified uterine endometrial stromal cells. The process of decidual cell formation or decidualization is characteristic of hemochorial placentation found in primates and rodents (Parr and Parr, 1989). During gestation, decidual cells are located at the interface separating invading trophoblast cells from the maternal environment. A number of important functions have been attributed to decidua (Bell, 1983): 1) a protective role in controlling trophoblast cell invasion; 2) a nutritive role for the developing embryo; 3) a role in preventing immunological rejection of genetically disparate embryonic/fetal tissues; and 4) an endocrine/paracrine role in controlling maternal adaptations required for the establishment and maintenance of pregnancy. Pregnancy is dependent upon decidual cell acquisition of each of these specialized functions. The ovaries and blastocyst provide signals responsible for initiating changes in the uterus. Differentiation of decidual cells is among the earliest uterine adaptations to pregnancy (Parr and Parr, 1989) and is exquisitely sensitive to the regulatory actions of progesterone (Brar et al., 1997). Decidual cells have profound effects on the local uterine environment (Parr and Parr, 1989). The uterus shows dramatic changes in its vascularization and the distribution and function of its immune and inflammatory cell constituents following decidualization (Parr and Parr, 1989).

Trophoblast. Trophoblast cells are the parenchymal cells of the placenta. They are specialized, exhibit distinct phenotypes, and arise via a multilineage differentiation process (Gardner and Beddington, 1988). Trophoblast lineages go on to contribute to the formation of the chorioallantoic placenta (Enders and Welsh, 1993). These structures are responsible for controlling fetal and maternal environments during pregnancy. The chorioallantoic placenta is organized into components that specialize in invasion, endocrine activities, and bidirectional transport.

Pregnancy-Specific Modulators

Decidual and trophoblast cells secrete an assortment of regulatory molecules that can be variously classified as chemokines, cytokines, growth factors, and hormones. These agents modulate the maternal environment making it more amenable to pregnancy. We can identify three categories of pregnancy-specific modulators secreted by decidual and/or trophoblast cells: 1) common regulatory molecules controlled by pregnancy-specific signals; 2) unique regulatory molecules which are mimics of known ligands; 3) unique regulatory molecules with apparently unique actions. Other common regulatory molecules controlled by common tissue-nonspecific signals may also contribute to the establishment and maintenance of pregnancy but will not be discussed in this section. In the following paragraphs we address and provide examples for each category of pregnancy-specific modulator. Our ensuing discussion is meant to be illustrative and does not represent a complete list of all modulators. We utilize examples for immune and nonimmune regulatory molecules from primate, ruminant, and/or rodent model systems.

Common Regulatory Molecules Controlled by Pregnancy-Specific Signals. Both decidual and trophoblast

cells express genes encoding for hormones and/or enzymes involved in hormone biosynthesis, which are also expressed by other tissues. Most importantly, the regulation of these genes within decidual and trophoblast cells can be unique. This strategy has been utilized repeatedly. Examples include decidual cell expression of prolactin (PRL; Telgmann and Gellersen, 1998) and trophoblast cell expression of the aromatase enzyme (Kamat et al., 1998). In the nonpregnant state, PRL is a key product of the anterior pituitary, and aromatase is a key component of the estrogen biosynthetic pathway in the ovary. In both instances, decidual and trophoblast cells utilize distinct transcriptional control mechanisms, including usage of alternate promoters. Thus, common regulatory proteins can be brought under unique sets of control via reorganization of the transcriptional machinery present within cells situated at the maternal-fetal interface. Actions of common ligands may also be expanded or redirected via decidual and/or trophoblast cell-specific post-transcriptional or post-translational processing.

Unique Regulatory Molecules Acting As Mimics for Known Ligands. Both decidua and placenta produce hormones that are not generally produced by other tissues. This category includes unique ligands, which mimic the actions of ligands produced by other tissues. The most common examples are the hormones/cytokines, chorionic gonadotropin (CG), and placental lactogen (PL) of human pregnancy (Ogren and Talamantes, 1994) and interferon- γ (IFN- γ) of ruminant pregnancy (Roberts et al., 1999). These ligands are encoded by distinct genes yet interact with receptor-signaling pathways utilized by other ligands (CG, luteinizing hormone receptor; PL, PRL receptor; IFN- γ , type I IFN receptor). Controls for the genes encoding these ligands are unique to the trophoblast lineage. In addition to the site of synthesis, these trophoblast-specific ligands differ in their primary sequence and post-translational processing. These features may optimize their delivery to their respective targets and the resulting biological response. Although, not fully appreciated, it is important to recognize that mimics may act on their target cells differently than the ligands they mimic and may even possess distinct targets and actions.

Unique Regulatory Molecules with Apparently Unique Actions. A second group of unique regulatory molecules produced by decidua and placenta include those that appear to possess unique biological actions. The group is typified by the PRL family (Soares and Linzer, 2001). In rodents, the PRL family has undergone considerable expansion. Over two dozen genes, encoding for ligands with structural relationships to PRL, are expressed in decidual and trophoblast cells. A small subset of these ligands acts as mimics of PRL, whereas the majority does not utilize the PRL receptor-signaling pathway. Instead, they act on distinct targets via distinct mechanisms. These are hormones of pregnancy and their targets include the vasculature, hematopoietic cells, and intrauterine immune and inflammatory cells (Soares and Linzer, 2001). The mechanisms of action of many members of the PRL family are yet to be fully elucidated. A potentially exciting feature of at least one member of the PRL family, proliferin, is its reactivation during pathological states. During pregnancy, proliferin expression is restricted to trophoblast giant cells where it acts to promote blood vessel development; however, creation of a wound in the skin results in a dramatic increase in proliferin biosynthesis

where it is hypothesized to participate in the healing process (Fassett and Nilsen-Hamilton, 2001). Whether reactivation of decidual- and/or placental-specific ligands is a general feature of responses to pathology remains to be determined.

Overview of Pregnancy-Specific Modulators

Even a cursory examination of signaling mechanisms at the maternal-fetal interface indicates various levels of species diversity. Problems associated with young developing within the female reproductive tract are similar for all species. Adequate supplies of nutrients must be delivered to the embryo/fetus without compromising the mother. However, the solutions utilized by individual species vary widely. Among viviparous species there are striking differences in the organization of the maternal-fetal interface, the length of gestation, and the progression of embryonic/fetal development. Hence, it is not surprising that factors controlling the gestational state differ in a species-dependent manner. Functional homologies among species will exist and may include the ligands, their cellular targets, and/or components of their signaling pathways. As we learn more about pregnancy-specific modulators, new physiological mechanisms and molecular targets will be revealed. These efforts will lead to important opportunities for the design of therapeutics to specifically treat the mother or her developing fetus.

Multidrug-Resistant Transporters of the Placenta

Placental Multidrug-Resistant Gene Product 1 (MDR1) or P-glycoprotein (Pgp). The transporting Pgps are generally over-expressed in tumor cells, conferring resistance against cytotoxic agents, and are associated with specialized normal tissue barriers including the blood-brain barrier, gastrointestinal epithelium, blood-testis barrier, blood-ovarian barrier, and the placenta. In general, Pgp specifically refers to the product of MDR1 and is a large (140–170 kDa) ATP-dependent, inducible, and polyspecific transporter inserted in the plasma membrane that mediates the active efflux of substances out of the cell (Gottesman et al., 1996; Schinkel et al., 1996). In humans, the drug transporting Pgp is the product of the MDR1 gene. In rodents, two Pgp transporters include products of MDR1a (predominant in brain, liver, intestine, placenta) and MDR1b genes, respectively. The Pgps, specifically MDR1 and MDR1a, function in a similar manner, recognize a structurally and functionally diverse group of drugs, and show qualitatively similar but not identical substrate selectivity and affinities (Gottesman et al., 1996; Schinkel et al., 1996). Only the human MDR1, and MDRs 1 and 3 in the rodent have been shown to be involved in drug efflux (Fardel et al., 1996).

In the placenta of mice, trophoblasts appear to express a functional Pgp throughout pregnancy (Lankas et al., 1998). In the human placenta, there is abundant expression of the MDR1 gene throughout pregnancy (Mylona et al., 1996; Allikmets et al., 1998) and specifically in the cytotrophoblast (Mylona et al., 1996; Nakamura et al., 1997). Studies with microvillar membranes of term human trophoblasts, primary cultures of human cytotrophoblasts, and the BeWo cell line have confirmed expression of an active MDR1 (Nakamura et al., 1997; Utoguchi et al., 2000).

Lankas et al. (1998) provided evidence of the potential

functional importance of the placental Pgp in drug and chemical exposure using the spontaneous MDR1a “knockout” mouse, the outbred CF-1 strain in which MDR1a is naturally absent in 25% of the animals. Fetal localization of Pgp in normal and heterozygote mice was demonstrated on the surface of trophoblasts in the placental labyrinth, on the apical surface of endodermal cells of the yolk sac, and endothelial cells of the fetal brain. MDR1a was not present in placental cells of normal, heterozygote, or the knockout mice. The fetuses from the homozygous knockouts had a 100% susceptibility to chemically induced cleft palate compared with a 0% incidence in mice with normal MDR1a expression in the placenta. Heterozygotes fell in between in regards to susceptibility and incidences of cleft palate. In addition, the absence of placental MDR1a was correlated directly with actual chemical uptake by the fetus, at least a 5-fold greater uptake after a single dose and affirmed the placental role in *reducing* the chemical exposure burden to the fetus. More recently, Smit et al. (1999) have supported the study of Lankas et al. (1998), demonstrating in knockout mouse dams receiving intravenous drugs that are substrates of Pgp, fetuses were exposed to 2.4- to 16-fold increases in drug concentrations relative to fetuses in dams with normal placental Pgp expression. Thus, the Lankas et al. (1998) and Smit et al. (1999) studies illustrate the potential of the placental Pgp as a mechanism for limiting fetal chemical exposure, to possible teratogens, and other adverse effects of xenobiotics. Although natural Pgp knockouts can also be found in certain canine breeds, a similar natural MDR1 knockout has not as yet been observed in the human population (Lankas et al., 1997, 1998). There is evidence of functional polymorphisms of MDR1 in humans, and researchers are only now beginning to attempt correlations between expression and activity (Hoffmeyer et al., 2000; Tanabe et al., 2001).

Regulation and Structure-Activity Functions of Pgp.

In general, the control of functional Pgp expression is not precisely known in any cell type. Aside from certain xenobiotics (e.g., phenobarbital, reserpine, rifampicin) (Schuetz et al., 1996), it is known that endogenous steroid hormones (e.g., progesterone and estrogen) can induce Pgp in a variety of cell types (Jancis et al., 1993; Rao et al., 1994), including the Pgp in some tissues of pregnancy. MDR1 has been found in high levels in the uterine secretory epithelium and is apparently induced by estrogen and progesterone, the major steroid hormones of pregnancy (Arceci et al., 1990). Progesterone specifically is a potent inhibitor of Pgp, but not transported by the protein (Ueda et al., 1992; Barnes et al., 1996). Progesterone is also produced by granulosa cells and was shown to induce expression of Pgp in preovulatory follicles and in cells of corpora lutea in the rat. In this latter report, it was postulated that progesterone might modulate steroid efflux in these tissues (Lee et al., 1998). The few studies that exist seem to suggest that progesterone interacts directly with MDR1 and MDR1a proteins to effect a change in efflux (Shapiro et al., 1999). For the MDR1b rodent form, however, progesterone has been shown to regulate activity of this Pgp isoform at the gene promoter level (Piekarz et al., 1993). Despite the absence of evidence, one also cannot rule out a role for the glucocorticoid receptor in regulation of MDR1. Glucocorticoid receptor agonists are effective modulators of Pgp function and the similarity of structure-activity relation-

ships would argue for a possible physiological significance (Gruol and Bourgeois, 1997).

Information on MDR1-mediated chemical structure/efflux relationships is minimal in part due to the fact that this phenomenon has only been appreciated in recent years (Schinkel et al., 1996). MDR1 is well known to be "promiscuous" or polyspecific, interacting with structurally diverse drugs with the common feature of high lipophilicity and a preference for cationic amphiphilic chemicals (Smit et al., 1998). The polyspecific nature of MDR1 has been confirmed in transfected cell systems (Smit et al., 1998). In a survey of 100 different compounds, a pattern of recognition by MDR1 in a variety of cell types suggests that structure recognition by Pgp appears to be associated with the number of and proximity of electron donor or hydrogen bonding groups. The hypothesis holds that those compounds with certain fixed spatial distances between these chemical groups can be reasonably predicted as MDR1 substrates (Seelig, 1998; Seelig et al., 2000). As an additional consideration, multiple binding sites have also been proposed on MDR1, which could account for the polyspecificity of the protein. Shapiro et al. (1999) and Martin et al. (2000) proposed at least two transporting binding sites and one or more allosteric regulatory binding sites are present on MDR1. One of the regulatory sites binds progesterone and prazosin (Shapiro et al., 1999) and is implicated in the observations of steroid regulation of Pgp in the tissues of pregnancy. Therefore, investigation and development of an understanding of the underlying mechanisms by which progesterone or other steroid hormones regulate Pgp might result in strategies to pharmacologically manipulate efflux mechanisms to improve the safety and efficacy of certain therapeutics during pregnancy.

Other Multidrug-Resistant Transporters of the Placenta. Although widely distributed in epithelium and endothelium, alternative efflux proteins, lung resistance protein (LRP) and multidrug resistance-associated protein (MRP), have not been routinely observed in the human placenta (Sugawara et al., 1997). An early study (Flens et al., 1996) had suggested evidence of MRP expression in the human placenta. Subsequent studies suggest there are around seven MRPs known (Kool et al., 1997, 1999; Borst et al., 2000), and the most recent survey with more sensitive molecular techniques indicated at least three different MRP mRNAs are expressed in the human placenta (St-Pierre et al., 2000). Although MRPs generally prefer to transport organic anion drugs, metallic oxyanions, glutathione conjugates, including peptidyl leukotrienes, and glucuronates, uronates, sulfates, and organic anionic dyes, the physiological function of these transporters also remains unknown. MRP activity is inhibited by agents that inhibit the transport of organic anions such as probenecid (Barrand et al., 1997; Borst et al., 2000). MRP1, considered the major MRP isoform for drug efflux, and MDR1 share only a 15% homology in amino acid se-

quence. MRP is functional in the human syncytiotrophoblast vesicles using dinitrophenyl-glutathione, a conjugate substrate recognized by either MRP1 or MRP2 (St-Pierre et al., 2000) and in the cell line BeWo with unconjugated bilirubin as a substrate (Pascolo et al., 2001). However, MRP2 (also known as cMOAT) was the major form described in the syncytiotrophoblast with MRP1 and MRP3 being more predominantly confined to the blood vessel endothelia (St-Pierre et al., 2000). The mRNA for MRP5 is expressed in the human trophoblast, however, functional activity has not been demonstrated to date (Pascolo et al., 2001). MRP5 shows a preference in transporting nucleotide analogs, the anticancer drugs, 6-mercaptopurine, thioguanine, and the anti-HIV drug, 9-(2-phosphonylmethoxyethyl) adenine (Wijnholds et al., 2000).

Breast cancer resistance protein (BCRP/MXR/ABCP), a member of the same ATP binding cassette family of transporters that includes MDR1 and MRP was shown to be expressed and functional in mouse placenta. The presence or absence of MDR1 had no effect on expression or functional activity of BCRP1. BCRP1 likely reduces fetal exposure to some drugs (e.g., topotecan) in a manner analogous to MDR1 (Jonker et al., 2000). At present, little is known of the substrate specificity of BCRP1 or the overall significance of this protein relative to MDR1 and MRP in multidrug resistance (Jonker et al., 2000) or whether the transporter is associated with the human placenta.

Significance of Multidrug-Resistant Transporters at the Placenta. A better understanding of placental physiology and biochemistry is expected to lead to pharmacological approaches in controlling the possible fetal distribution of drugs administered in pregnancy (Audus, 1999). The accumulating evidence of multidrug-resistant transporters in the placenta as summarized in Table 1 provides a basis for suggesting that mechanisms might be targeted to facilitate safe and effective use of drugs in pregnancy. For example, the observations of polyspecificity and modulation by regulatory binding sites suggests that one can either design drug molecules that are not substrates for Pgp or one might pharmacologically stimulate Pgp through regulatory mechanisms for the benefit of protecting the fetus from xenobiotics. The possible significance of steroid hormone regulation of Pgp remains a question.

Although Pgp is currently viewed as the dominant efflux mechanism of the placenta, the observations of MRPs and BCRP presence in the tissue reveals multiple mechanisms that could eventually be targets for pharmacological manipulation and/or to drive design of safer drugs for pregnancy. The feasibility of taking advantage of placental transport mechanisms is demonstrated in a recent report by Pascual et al. (2001) describing the coupling of the anticancer drug cisplatin to a bile acid moiety. The cisplatin conjugate is prevented from passage across the placental barrier by a

TABLE 1
Evidence for multidrug-resistant transporters in the human placenta

Transporter	Location	Reference(s)
MDR1 or P-glycoprotein	Trophoblasts; trophoblastic cell line	Mylona et al., 1996; Nakamura et al., 1997; Allikmets et al., 1998; Utoguchi et al., 2000
MRP1 and MRP2	Syncytiotrophoblast; trophoblastic cell line	St-Pierre et al., 2000; Pascolo et al., 2001
MRP1 and MRP3 (expression only)	Blood vessel endothelia	St-Pierre et al., 2000
MRP5 (expression only)	Trophoblastic cell line	Pascolo et al., 2001

glycocholic acid transporter that transports glycocholic acid preferentially from fetus to the mother.

Conclusions

Characterization of cell-modulating factors at the maternal-fetal interface provides a number of potential therapeutic targets to intervene to improve the quality and success of pregnancy. Discussion here indicates that a fundamental understanding of the passive and active mechanisms regulating expression of class I molecules and the conferred protection against maternal immune cells may provide therapeutic targets. Specifically, strategies to prevent maternal rejection of fetal tissue due to dysregulation of HLA expression. There are opportunities for investigation of the fundamentals of the unique regulation of hormones and enzyme systems originating from decidua and trophoblast cells by pregnancy-specific modulators. Knowledge of these modulators and their function could be used to correct corrupted signaling that would otherwise prevent normal embryo implantation and fetal development. Finally, knowledge of the functions and hormonal regulation of multidrug-resistant transporters at the placenta may offer the possibility of future design and development of drugs for use in pregnancy that have higher benefit and lower risk for both mother and fetus.

References

- Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, and Dean M (1998) A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Res* **58**:5337–5339.
- Arceci RJ, Baas F, Raponi R, Horwitz SB, Housman D, and Croop JM (1990) Multidrug resistance gene expression is controlled by steroid hormones in the secretory epithelium of the uterus. *Mol Reprod Dev* **25**:101–109.
- Audus KL (1999) Controlling drug delivery across the placenta. *Eur J Pharm Sci* **8**:161–165.
- Barnes KM, Dickstein B, Cutler GB Jr, Fojo T, and Bates SE (1996) Steroid treatment, accumulation, and antagonism of P-glycoprotein in multidrug-resistant cells. *Biochemistry* **35**:4820–4827.
- Barrand MA, Bagrij T, and Neo S-Y (1997) Multidrug resistance-associated protein: a protein distinct from P-glycoprotein involved in cytotoxic drug expulsion. *Gen Pharmacol* **28**:639–645.
- Bell SC (1983) Decidualization: regional differentiation and associated function. *Oxford Rev Reprod Biol* **5**:220–271.
- Borst P, Evers R, Kool M, and Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* **92**:1295–1302.
- Brar AK, Frank GR, Kessler CA, Cedars MI, and Handwerker S (1997) Progesterone-dependent decidualization of the human endometrium is mediated by cAMP. *Endocrine* **6**:301–307.
- Croy BA, Whitelaw PF, and Engelhardt H (1998) The influences of immune cells on the success of pregnancy, in *The Endocrinology of Pregnancy* (Bazer FW ed) pp 229–289, Humana Press, Inc., Totowa, NJ.
- Enders AC and Welsh AO (1993) Structural interactions of trophoblast and uterus during hemochorial placenta formation. *J Exp Zool* **266**:578–587.
- Fardel O, Lecœur V, and Guillouzo A (1996) The P-glycoprotein in multidrug transporter. *Gen Pharmacol* **27**:1283–1291.
- Fassett JT and Nilsen-Hamilton M (2001) Mrp3, a mitogen-regulated protein/proliferin gene expressed in wound healing and in hair follicles. *Endocrinology* **142**:2129–2137.
- Flens MJ, Zaman GJ, van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, van der Groep P, de Haas M, Meijer CJ, and Scheper RJ (1996) Tissue distribution of the multidrug resistance protein. *Am J Pathol* **148**:1237–1247.
- Fournel S, Aguerre-Girr M, Huc X, Lenfant F, Alam A, Toubert A, Bensussan A, Le Bouteiller P (2000) Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis. *J Immunol* **164**:6100–6104.
- Gardner RL and Beddington RSP (1988) Multi-lineage stem cells in the mammalian embryo. *J Cell Sci Suppl* **10**:11–27.
- Geraghty DE (1993) Structure of the HLA class I region and expression of its resident genes. *Curr Opin Immunol* **5**:3–7.
- Gottesman MM, Pastan I, and Ambudkar SV (1996) P-glycoprotein and multidrug resistance. *Curr Opin Genet Dev* **6**:610–617.
- Gruol DJ and Bourgeois S (1997) Chemosensitizing steroids: glucocorticoid receptor agonists capable of inhibiting P-glycoprotein function. *Cancer Res* **54**:720–727.
- Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, Casacorb I, Gerloff T, Roots I, Eichelbaum M, and Brinkmann U (2000) Functional polymorphisms of the human multidrug resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci USA* **97**:3473–3478.
- Hunt JS, Fishback JL, Andrews GK, and Wood GW (1988) Expression of class I HLA genes by trophoblast cells: analysis by *in situ* hybridization. *J Immunol* **140**:1293–1299.
- Hunt JS, Fishback JL, Chumbley G, and Loke YW (1990) Identification of class I MHC mRNA in human first trimester trophoblast cells by *in situ* hybridization. *J Immunol* **144**:4420–4425.
- Hunt JS and Orr HT (1992) HLA and maternal-fetal recognition. *FASEB J* **6**:2344–2348.
- Ishitani A and Geraghty DE (1992) Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. *Proc Natl Acad Sci USA* **89**:3947–3951.
- Jancis EM, Chen HX, Carbone R, Hochberg RB, and Dannies PS (1993) Rapid stimulation of rhodamine 123 efflux from multidrug-resistant KB cells by progesterone. *Biochem Pharmacol* **46**:1613–1619.
- Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JHM, and Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* **92**:1651–1656.
- Kamat A, Alcorn JL, Kuncz C, and Mendelson CR (1998) Characterization of the regulatory regions of the human aromatase (P450arom) gene involved in placenta-specific expression. *Mol Endocrinol* **12**:1764–1777.
- Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, and Borst P (1997) Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res* **57**:3537–3547.
- Kool M, van der Linden PA, de Haas M, Baas F, and Borst P (1999) Expression of human MRP6, a homologue of the multidrug resistance protein gene MRP1, in tissues and cancer cells. *Cancer Res* **59**:175–182.
- Lankas GR, Cartwright ME, and Umbenhauer D (1997) P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. *Toxicol Appl Pharmacol* **146**:88–94.
- Lankas GR, Wise LD, Cartwright ME, Pippert T, and Umbenhauer DR (1998) Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. *Reprod Toxicol* **12**:457–463.
- Le Bouteiller P (1996) HLA class I genes and products, in *HLA and the Maternal-Fetal Relationship* (Hunt JS ed) pp 51–85, R. G. Landes Publishing Company, Austin, TX.
- Le Bouteiller P and Mallet V (1997) HLA-G and pregnancy. *Rev Reprod* **2**:7–13.
- Le Bouteiller P and Solier C (2001) Is antigen presentation the primary function of HLA-G? *Microbes Infect* **3**:323–332.
- Lee GY, Croop JM, and Anderson E (1998) Multidrug resistance gene expression correlates with progesterone production in dehydroepiandrosterone-induced polycystic and equine chorionic gonadotropin-stimulated ovaries of prepubertal rats. *Biol Reprod* **58**:330–337.
- Long EO (1999) Regulation of immune responses through inhibitory receptors. *Ann Rev Immunol* **17**:875–904.
- Martin C, Berridge G, Higgins CF, Mistry P, Charlton P, and Callaghan R (2000) Communication between multiple drug binding sites on P-glycoprotein. *Mol Pharmacol* **58**:624–632.
- Medawar PB (1953) Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol* **7**:320–338.
- Murphy SP and Tomasi TB (1998) Absence of MHC class II antigen expression in trophoblast cells results from a lack of class II transactivator (CIITA) gene expression. *Mol Reprod Dev* **51**:1–12.
- Mylona P, Glazier JD, Greenwood SL, Slides MK, and Sibley CP (1996) Expression of the cystic fibrosis (CF) and multidrug resistance (MDR1) genes during development and differentiation in the human placenta. *Mol Hum Reprod* **2**:693–698.
- Nakamura Y, Ikeda S, Furukawa T, Sumizawa T, Tani A, Akiyama S, and Nagata Y (1997) Function of P-glycoprotein expressed in placenta and mole. *Biochem Biophys Res Commun* **235**:849–853.
- Ober C, Aldrich C, Rosinsky B, Robertson, Walker MA, Willadsen S, Verp MS, Geraghty DE and Hunt JS (1998) HLA-G1 protein expression is not essential for fetal survival. *Placenta* **19**:127–132.
- Ogren L and Talamantes F (1994) The placenta as an endocrine organ: polypeptides, in *The Physiology of Reproduction, Second Edition* (Knobil E and Neill JD eds), pp 875–945, Raven Press, New York.
- Parr MB and Parr EL (1989) The implantation reaction, in *Biology of the Uterus* (Wynn RM and Jollie WP eds), pp 233–278, Plenum, New York.
- Pascolo L, Ferneti C, Garcia-Mediavilla MV, Ostro JD, and Tiribelli C (2001) Mechanisms for the transport of unconjugated bilirubin in human trophoblastic BeWo cells. *FEBS Lett* **495**:94–99.
- Pascual MJ, Macias RI, Garcia-Del-Pozo J, Serrano MA, and Marin JJ (2001) Enhanced efficiency of the placental barrier to cisplatin through binding to glycocholic acid. *Anticancer Res* **21**:2703–2707.
- Piekarczyk RL, Cohen D, and Horwitz SB (1993) Progesterone regulates the murine multidrug resistance mdr1b gene. *J Biol Chem* **268**:7613–7616.
- Rao US, Fine RL, and Scarborough GA (1994) Antiestrogens and steroid hormones: Substrates of the human P-glycoprotein. *Biochem Pharmacol* **48**:287–292.
- Roberts RM, Ealy AD, Alexenko AP, Han CS, and Ezashi T (1999) Trophoblast interferons. *Placenta* **20**:259–264.
- Sanders SK, Giblin PA, and Kavathas P (1991) Cell-cell adhesion mediated by CD8 and human histocompatibility leukocyte antigen G, a nonclassical major histocompatibility complex class I molecule on cytotrophoblasts. *J Exp Med* **174**:737–740.
- Schinkel AH, Wagenaar E, Mol CAAM, and van Deemter L (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* **97**:2517–2524.
- Schuetz EG, Beck WT, and Schuetz JD (1996) Modulators and substrates of P-glycoprotein and cytochrome P450 3A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* **49**:311–318.
- Seelig A (1998) A general pattern for substrate recognition by P-glycoprotein. *Eur J Biochem* **251**:252–261.
- Seelig A, Blatter XL, and Wohnsland F (2000) Substrate recognition by P-

- glycoprotein and the multidrug resistance-associated protein MRP1: A comparison. *Int J Clin Pharmacol Ther* **38**:111–121.
- Shapiro AB, Fox K, Lam P, and Ling V (1999) Stimulation of P-glycoprotein-mediated drug transport by prazosin and progesterone. *Eur J Biochem* **259**:841–850.
- Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, and Schinkel AH (1999) Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *J Clin Invest* **104**:1441–1447.
- Smit JW, Weert B, Schinkel AH, and Meijer DKF (1998) Heterologous expression of various P-glycoproteins in polarized epithelial cells induces directional transport of small (type 1) and bulky (type 2) cationic drugs. *J Pharmacol Exp Ther* **286**:321–327.
- Soares MJ and Linzer DIH (2001) Rodent prolactin family and pregnancy, in *Prolactin* (Horseman ND ed), pp 139–167, Kluwer Academic Publishers, Norwell, MA.
- St-Pierre MV, Serrano MA, Macias RIR, Dubs U, Hoechli M, Lauper U, Meier PJ, and Marin JJG (2000) Expression of members of the multidrug resistance protein family in human term placenta. *Am J Physiol* **279**:R1495–R1503.
- Sugawara I, Akiyama S, Scheper RJ, and Itoyama S (1997) Lung resistance protein (LRP) expression in human tissues in comparison with that of MDR1 and MRP. *Cancer Lett* **112**:23–31.
- Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, and Otsubo K (2001) Expression of P-glycoprotein in human placenta: Relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Exp Pharmacol Ther* **297**:1137–1143.
- Telgmann R and Gellersen B (1998) Marker genes of decidualization: activation of the decidual prolactin gene. *Human Reprod Update* **4**:472–479.
- Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioda N, Komano T, and Hori R (1992) Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* **267**:24248–24252.
- Utoguchi N, Chandorkar GA, Avery M, and Audus KL (2000) Functional expression of P-glycoprotein in primary cultures of human cytotrophoblasts and BeWo cells. *Reprod Toxicol* **14**:217–224.
- Wijnholds J, Mol CAAM, van Deemter L, de Haas M, Scheffer GL, Baas F, Beijnen JH, Scheper RJ, Hatse S, De Clercq E, Balzarini J, and Borst P (2000) Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleotide analogs. *Proc Natl Acad Sci USA* **97**:7476–7481.
- Zavazava N (1998) Soluble HLA class I molecules: biological significance and clinical implications. *Mol Med Today* **4**:116–121.

Address correspondence to: Dr. Kenneth L. Audus, Department of Pharmaceutical Chemistry, The University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047-3729. E-mail: audus@ku.edu
