Commentary

The Placenta Revealed

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While the emergence of the diagnostic biopsy in the 1940s has helped to elucidate the pathophysiology of most organs, our understanding of the placenta has, until recently, lagged behind. With little opportunity for serial biopsies, the placenta is most often examined only at the end of a gestation, hampering our understanding of the dynamic, progressive nature of diseases of pregnancy involving the placenta. Now, a more basic understanding of trophoblast biology and the application of molecular biological tools to this clinical material are beginning to advance the level of knowledge for placental pathologists. Where once we could only speculate about the pathological processes taking place during gestation, we are beginning to fit the pieces together into a more global understanding of the dynamic relationships between the mother and placenta during pregnancy.

Three areas of research are converging on the placenta: basic trophoblast biology, the reproductive immunology of the uteroplacental unit, and infectious agent identification. As the tools in each of these areas have advanced, their application to problems in placental pathology has helped to sort out the causes and effects of a number of important diseases of pregnancy, including preeclampsia, intrauterine growth retardation, and infection.

Advances in Trophoblast Biology

Recent research has uncovered the pathways of trophoblast differentiation from compartment to compartment within the uteroplacental unit. Two types of trophoblasts have been traditionally described: the cytotrophoblast and the syncytiotrophoblast. With the development of reproducible methods of trophoblast culture,[1] improved markers

of trophoblast synthetic activity,[2] and a deeper understanding of the functions that trophoblasts play in the utero-placental unit,[3-7] we have now been able to identify more specific subsets of trophoblasts. These include the undifferentiated mononuclear precursor of all trophoblast forms, the cytotrophoblast; the endocrinologically active villous syncytiotrophoblast; the junctional trophoblast that attaches the anchoring villi to the maternal decidua at Nitabuch's layer; and the invasive intermediate trophoblast that migrates into the decidua, the myometrium, and finally the spiral arteries of the uterus.[2]

Cytotrophoblasts, the stem cells of all other trophoblast forms, can be most easily identified in the chorionic villi throughout gestation as the large mononuclear cells that separate the villous basement membrane from the overlying syncytial trophoblast layer. Although not always clearly identifiable with hematoxylin and eosin-stained sections, cytotrophoblasts are particularly easy to recognize as negatively stained cells when the placenta is immunohistochemically stained for one of many syncytiotrophoblast markers (ie, human chorionic gonadotropin or human placental lactogen).[8] This is especially true at term when, in comparison to the first trimester, the number of cytotrophoblasts decreases. Purification and culture of cytotrophoblasts has demonstrated that these cells express few trophoblast markers at first, but over a period of several days they fuse to form syncytiotrophoblasts and acquire many differentiated trophoblast markers.[2]

Junctional trophoblasts are also derived from villous cytotrophoblasts. Examination of the villi adjacent to Nitabuch's layer at the placenta's attachment zone to the maternal decidua reveals a population of large, mononuclear trophoblasts, growing out of the villi, forming what has been

called the cell columns. Classic histopathologists have suggested that these trophoblasts anchor the placenta to the uterus. Until recently, however, the biochemistry and unique differentiation of these cells were not appreciated. It seems that these cells are engaged both in attachment and invasive functions. Feinberg et al[4] recently described a unique fibronectin—trophouteronectin—expressed by these cells. This fibronectin, which is one of the class of oncofetal fibronectins,[9] seems to be a specific marker of junctional trophoblasts because it is present at the uteroplacental junction, at the junction of the trophoblasts of the external membranes with maternal tissue, and at the junction of attaching trophoblasts and fallopian tube epithelium in tubal ectopic pregnancies. More recently, Zhou et al[7] have shown that the trophoblasts of these attachment zones express different integrins at the placental side compared to the maternal side of the column, suggesting a differentiation gradient between maternal and fetal environments. It is possible that one or more of the many growth factors and cytokines made by the decidua may be involved in the expression of both this unique form of trophoblast fibronectin and its associated integrins.[10–18] Finally, Fernandez et al have shown that these cells also express type IV collagenase, demonstrating that, in addition to anchoring, at least some of these trophoblasts are capable of extracellular matrix degradation, a necessary component for trophoblast invasion.[6]

The presence of invasive trophoblasts within the decidua and myometrium has been appreciated for some time,[19] but it is only recently that researchers have attributed specific markers, and hence, specific functional characteristics, to these cells. The first clear marker of the invasive trophoblast was described by Kurman and colleagues,[20] who demonstrated that first trimester invasive trophoblasts react with anti-human placental lactogen antibodies. They coined the term intermediate invasive trophoblast partly because of its intermediate size between cyto- and syncytiotrophoblasts. Feinberg et al[3] demonstrated that these same cells express plasminogen activator inhibitor type I, suggesting that intermediate invasive trophoblasts may utilize, in addition to the collagenases, the plasminogen activator system to perform their invasive function. More recently, Zhou et al[7] have shown that as trophoblasts leave the cell columns and enter the maternal space they lose integrins for basement membrane interactions (possibly laminin) and gain integrins for fibronectin and type I collagen interactions. In addition to the presence of markers of ex-

tracellular matrix interactions and proteases needed for cell movement and invasion, these trophoblasts also seem to express a unique monomorphic histocompatibility antigen: HLA-G.[21,22] Researchers think that this particular HLA serves to protect the trophoblast from maternal recognition and destruction by decidual large granular lymphocytes, a type of natural killer cell.[23,24]

Collectively, these data point to a complex array of factors that must be involved in the control of trophoblast differentiation.[2] This complexity may also help to explain the varied clinical diseases that can be seen during pregnancy. We are now beginning to appreciate that defects in trophoblast attachment or trophoblast invasion may be the basis of such clinical problems as faulty implantation (inadequate or defective trophouteronectin), early pregnancy loss (poor trophoblast invasion or loss of trophouteronectin), abruption (loss or down-regulation of integrins or trophouteronectin at the uteroplacental junction), preeclampsia (abnormal, inadequate, impeded trophoblast invasion, or immune rejection of invasive trophoblasts), placenta accreta (increased trophoblast invasiveness or increased expression of attachment factors), or even placental site tumor and choriocarcinoma (loss of inhibition of trophoblast invasiveness or decrease in maternal surveillance of invasive trophoblasts).

Reproductive Immunology

A fundamental question still dominates reproductive immunology: why doesn't the mother reject the placenta? For many years, the most we could do was to describe the presence or absence of various inflammatory cells within the decidua and placenta during pregnancy. Without specific markers it was often unclear what these cells were, where they came from, and what their function was in each location. In certain disease states of the placenta, mononuclear cells can be seen infiltrating the chorionic villi of the placenta. Until the work of Redline and Patterson[25] (described elsewhere in this issue), however, the origin of these cells has been controversial, some arguing for a fetal origin, some for a maternal origin. Immunochemistry alone could not answer this question. Their approach of using in situ hybridization for Y and X markers in male gestations is an excellent example of the use of molecular techniques to answer persistent problems in placental pathology. From their studies, we now know that the lymphocytes present in cases of villitis of unknown etiology (VUE) are maternally derived, allowing us to focus on the causes of this apparent

maternal immunological reaction against tropho-blast and/or villous antigens.

Another example of the recent identification of an important immune cell in the uteroplacental unit is that of the large granular lymphocyte (LGL). Based solely on hematoxylin and eosin sections, it was thought that the nonstromal cells present in the endometrium in the late luteal phase and in pregnancy were granulocytes.[26] With the aid of specific antibodies and cell-surface markers, we now know that many of these cells are CD56-positive LGLs, a natural killer-like cell.[27-29] LGLs seem to be bone marrow-derived cells that are chemoattracted into the endometrial stroma during the late luteal phase of the menstrual cycle[30] and continue to be found in the decidua during pregnancy.[31] In vitro studies suggest that LGLs are cytotoxic against trophoblasts[23,24] and therefore may serve to limit trophoblast invasion. The relationship between LGLs and trophoblasts seems to be complex because there is evidence that cytokines are necessary for the activation of LGLs,[32] that hormones[33] and placental proteins[34] may regulate LGL function, and that trophoblasts may escape LGL attack by expressing specific HLAs.[24,35] These studies are finally elucidating for us the mechanisms by which the placenta normally protects itself from maternal rejection and can give us a basis by which to treat women whose immune cells inappropriately recognize and attack the placenta.

Infectious versus Immunological Diseases of the Placenta

With the aid of highly specific antibodies and nucleic acid probes, many nonbacterial infectious diseases of the placenta that were previously not diagnosable can now be recognized. Human immunodeficiency virus,[36,37] cytomegalovirus,[38,39] herpes simplex virus,[40] parvovirus,[41] measles virus,[42] enterovirus,[43] and hepatitis B[44] have all been identified within cells of the placenta and/or decidua in affected pregnancies. Because many kinds of villidites are characterized by lymphocytic infiltration of the chorionic villi,[45] distinguishing between villitis of unknown etiology and infectious villitis can be difficult without special staining. But the distinction is vital. Because villitis of unknown etiology probably represents a maternal immunological reaction against fetal antigens—as suggested by the work of Redline and Patterson[25]—its treatment would be very different from potential treatments of

viral infections. Immunosuppressive therapies that might be useful in cases of intrauterine growth retardation caused by maternal rejection of the placenta would be clearly contraindicated in cases of viral infection of the placenta.

These studies have begun to elucidate the many cellular interactions that take place between the placenta and mother in normal and diseased pregnancies. Our next challenge will be to apply the insights we have been gaining about trophoblast biology and the immunology of the uteroplacental unit to patients with complications of pregnancy. Although it is helpful to know what *has* happened in the placenta, it is far better to know what *will* happen. With the advent of highly refined techniques that make placental and decidual blood flow visible,[46-48] we are approaching the time when we, like the liver and renal pathologists before us, may be able to suggest responsibly that placental and decidual biopsies during a gestation can be effective and safe tools to diagnose diseases of pregnancy.

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