Editorials

The Role of the Placenta in the Maintenance of Normal Pregnancy

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In the article "Immunodetection of living trophoblast" that appears in this issue of the Journal, David Peleg et al. [1] demonstrate that monoclonal antibodies to human chorionic gonadotropin specifically bind to syncytiotrophoblastic cells of first trimester human placenta. This immunostaining method can be used to detect human trophoblastic cells in normal and pathological conditions. It can be used to rapidly demonstrate the presence of chorionic villi in human abortions as well as to demonstrate choriocarcinoma cells or any remnants of intact trophoblastic cells.

Trophoblastic cells first start to develop by the end of the first week post-fertilization when the early embryo develops into the inner cell mass and the outer trophoblastic layer [2,3]. Very rapidly the trophoblastic cells differentiate into two layers of cells, the outer syncytium and the inner cytotrophoblast, the syncytiotrophoblast invading the decidua to establish the appropriate contact between the embryo and the maternal uterus. Almost from the beginning the function of these two types of trophoblastic cells differ. The syncytiotrophoblastic cells synthesize a variety of proteolytic and other enzymes to enable their invasion into the decidua and develop adequate embryonic histiotrophic nutrition. In addition, they contain the machinery for the secretion of various hormones necessary for pregnancy maintenance and proper embryonic development. The main role of the cytotrophoblastic cells is to proliferate, mature and fuse to form the functional syncytiotrophoblastic cells [2,3]. Since the syncytiotrophoblastic cells are those that secrete various proteins and hormones, antibodies aimed against those proteins, such as anti-hCG antibodies, will specifically bind to these cells. Peleg et al. suggest that the monoclonal anti-hCG antibody is "capturing" hCG molecules as they are secreted from the trophoblastic cells. Alternatively, the antibodies bind to hCG, which is bound to hCG receptors, known to exist on the surface of the trophoblastic cell but not necessarily implying that the hCG is secreted by the same cell.

Any agent that would interfere with differentiation of cytotrophoblast to syncytiotrophoblast could inhibit normal placentation and hence embryonic development. Antiphospholipid antibodies have been shown to inhibit differentiation of cytotrophoblastic cells to syncytium, often resulting in impaired pregnancy and in spontaneous abortions [4].

The role of the placenta in immunological protection of the embryo

A very important role of syncytiotrophoblastic cells is thus their function in pregnancy maintenance. The developing embryo constitutes an organism with foreign antigens for the mother. It was initially believed that the semi-allogeneic conceptus withstands rejection because the syncytiotrophoblastic cells lack the expression of classical major histocompatibility complex antigens on their surface, thus attributing to the syncytiotrophoblastic cells a major role in embryonic protection [4]. This is no longer considered a major mechanism since anti-paternal, anti-embryonic and anti-placental antibodies have been found in sera from women with successful pregnancies [4,5]. Tolerance is believed to result from the interaction of various cytokines secreted by maternal and fetal cells, as well as progestational hormones secreted by the placenta. It is also believed that progesterone initiates the production by maternal lymphocytes of a progesterone-induced blocking factor, which protects the embryo by suppressing mitogen and antigen-induced lymphocyte proliferation and natural killer cell activity [5]. This substance apparently blocks the production of tumor necrosis factor by NK cells and enhances the production by lymhocytes of interleukin 4 and 10, both being "embryonic protecting" substances [4,5].

The protection exerted by the developing placenta is not only a result of the secretion of progesterone that seems to initiate the process, but apparently is also due to the active secretion of placenta-derived suppressor factors whose

hCG = human chorionic gonadotropin

NK = natural killer

nature is not yet known. This factor(s) that is secreted by cultured human placental cells inhibits mitogen- and antigen-induced human and murine lymphocyte proliferation [5].

Antiphospholipid syndrome and recurrent abortions

Imbalance in the interactions of the various cytokines and substances described above may result in spontaneous abortions [4,5]. Such an imbalance may also exist in maternal immunological diseases such as systemic lupus erythematosus or antiphospholipid syndrome. APS is often characterized by recurrent abortions, which are attributed by many investigators to the direct effects of the antiphospholipid antibodies such as anticardiolipin, anti-β_2 glycoprotein I or antiphosphatidylserine [6–9].

The aPA seem to be dependent on β_2GPI, a 50 kDa glycoprotein present in the circulation, for their function. β_2GPI acts as an anticoagulant and is an important target for binding of autoimmune aPA [8–10]. Anti β_2GPI I probably also exert a direct pathogenic effect by interfering with homeostatic reactions occurring on the surface of vascular endothelial cells, inducing vascular thrombosis.

In contrast to this view implicating aPA in the etiology of spontaneous abortions in APS, some investigators now regard aPA as a marker for recurrent abortions and not necessarily as a primary cause. They claim that there is no conclusive evidence of a role of aPA in obstetric problems, particularly recurrent abortions [9–11]. The pathogenesis of these abortions continues to be poorly understood.

Placental pathology in APS

A possible pathogenic mechanism for spontaneous abortion in APS is placental pathology. Various studies have shown that in APS the aPA seem to affect the developing placenta. It has also been suggested [10–13] that the abortions that are induced by aPA are the result of placental thrombosis and infarcts which might be responsible for reduced uteroplacental blood flow.

Several investigators have observed decidual vasculopathy, thrombosis and infarcts of the placenta associated with fetal death in a large proportion of recurrent abortions in women with aPA. Delayed transformation of cytotrophoblast to syncytiotrophoblast and increased thromboxane production by the placenta, which can lead to thrombosis at the uteroplacental interface, was also observed [12,13]. The observed placental pathological (and functional) changes are thought to lead to fetal death, but may well be part of the damage associated with fetal death. An increase in hCG production by human placental explants was shown in placental villi cultured for 24 hours in medium containing mouse aCA, but human aCA caused a reduction in hCG secretion [14].

Studies in our laboratory

During the last few years we have been studying the effects of sera from women with SLE and/or APS who suffer from recurrent abortions on 10.5 day old rat embryos cultured in these sera, or on placental explants. We performed the following studies [15–18]:

- We studied the effects of sera from untreated women with SLE and/or APS and recurrent abortions on 10.5 day old rat embryos in culture, focusing on embryonic growth and development and on the structure and function of the yolk sac.
- We studied the effects of treatment in these women by culturing rat embryos in sera from women with SLE and/or APS successfully treated with glucocorticosteroids and aspirin, and examined the effects on the embryos and on their yolk sacs.
- We studied the effects of sera from women with SLE and/or APS with and without treatment on the growth and function of 5–10 weeks old placental explants in culture.

Embryo culture studies: Evaluation of the embryos cultured on sera from women with SLE and/or APS showed a high rate of embryonic death in the experimental embryos with reduced or absent yolk sac circulation. A high rate of anomalies was found in the living embryos cultured on serum from these women in comparison to controls. The living embryos were small and had a lower protein content. The rate of anomalies was significantly reduced following successful treatment in the women [15,16].

Studies on yolk sacs: Transmission electron microscopy studies of the yolk sacs revealed a decrease in the average number of microvilli and an increase in inclusions in the yolk sacs of embryos cultured in sera from untreated women with SLE and/or APS as compared to controls [15,16]. We also studied the endocytic functions of the yolk sacs (transfer of tritiated sucrose) and found a marked decrease in endocytic index in the yolk sacs of embryos cultured on serum from women with SLE and/or APS without treatment. The endocytic index of the yolk sacs from embryos cultured in sera from treated women was normal, resembling that of controls.

Studies on human placental explants: The results of the yolk sac examinations show that in women with SLE and/or APS the target for the injuries produced by aPA may be the placenta – i.e., the trophoblastic cells. We therefore undertook further studies on cultured human trophoblastic cells. Culturing 5–10 weeks old human placental villi in sera from

APS = antiphospholipid syndrome
aPA = antiphospholipid antibodies
anti-β_2 glycoprotein 1
aCA = anticardiolipin antibodies
SLE = systemic lupus erythematosus

untreated women with SLE and/or APS, we found, firstly, that it is possible to culture human placentae on 90% control human serum, and that in this culture medium the placental explants grow even better than in the regular culture medium (HAM + F12). Differences from controls were found in estradiol, progesterone and hCG secretion by the trophoblastic cells cultured on the sera from women with SLE and/or APS. However, it is difficult to interpret the results of these studies since the sera serving as culture had various levels of these hormones even before culture and these levels were not assessed by us [17,18].

We examined the growth of the placental explants in the various culture media and found decreased proliferation and increased apoptosis in placental explants grown on serum from women with SLE and APS (6.5% proliferation vs. 56.8% in controls, and 6.3% apoptosis vs. 2.8% in controls). Treatment improved trophoblastic proliferation rate and decreased apoptosis (proliferation rate 11.9% and apoptosis 3.9%), but the proliferation rate still remained significantly lower than in the placental explants cultured in control serum [17,18].

In conclusion, when spontaneous abortions occur as a result of immunological impairment in maternal-fetal immune relations, the placenta may be an important target for the pathological changes that lead to abortion. Direct measures of the extent of placental damage are poorly defined, and the studies described by Peleg and co-workers in this issue of the Journal may perhaps be extended to such pathological conditions. It may be possible to see whether under conditions causing spontaneous abortions the immunostaining by anti-hCG antibodies is similar to that found in normal trophoblast or not. Moreover, specific antibodies against other components of trophoblastic cells may also be used in order to broaden the potential use of this technique.

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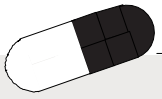
Capsule



Inserting a hairpin

Ticks harbor intriguing endosymbiotic bacteria with pared down genomes, called *Rickettsia*, which appear to be living relatives of mitochondria. Blood-feeding triggers replication of the bacteria, which are then injected into vertebrate hosts where they reside less benignly. Ogata et al. show that *Rickettsia conorii* harbors a distinctive repetitive insert in its genome that encodes a hairpin RNA. The insert appears to parasitize the open-reading frames that encode several conserved proteins and may lead to the evolution of new protein structures; a useful trick for an organism with a minimalist genome.

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