In vitro culture conditions, antiphospholipid antibodies and trophoblast function

Sir,

We read with interest the review of in vitro effects of antiphospholipid antibodies (aPL) on placental cells cultures by Tong et al. (2015). We congratulate the authors on this comprehensive evaluation of the literature to date. Dr Tong and colleagues conceded the limitations on their ability to draw conclusions, notably the heterogeneity of the studies including variable placental models, sources of aPL and effects measured. Despite these limitations, however, the authors concluded that aPL reduce trophoblast viability, sycnicalization and invasion in vivo and suggested that the cumulative data support the theory of aPL-mediated dysfunctional placental development in vivo. We propose there is still insufficient or unsubstantiated evidence that aPL interfere with early development of the placenta in vivo for several reasons. Disregarding for a moment the heterogeneity of the objectives of the studies cited, there are several issues with regard to the in vitro culture systems that concern us.

First, the reviewed studies used incubation of either first trimester or term placental explants, isolated trophoblasts, or choriocarcinoma cell lines. We appreciate the need for use of in vitro culture systems in the absence of a sufficiently representative animal model of placental development (Kaufmann et al., 2003). However, it would seem implausible that these diverse tissues all represent the in vivo milieu in the early stages of human pregnancy. Term placental explants in particular have been reported as unsuitable for investigation of early first trimester physiology, due to the lack of a placental bed (Kaufmann et al., 2003).

Second, the in vitro culture systems used by many if not all investigators included in this review used atmospheric O2 concentrations: six randomly selected papers from among those cited utilized 95% air with 5% CO2. Atmospheric air contains between 20–21% O2 as opposed to 3–5% O2 reportedly found in situ in the uterus (Ivanovic, 2009). It has been suggested that O2 concentration in tissue culture should closely approximate in vivo conditions to result in reliable insight into cell physiology (Ivanovic, 2009; Carreau et al., 2011). Even the action of low molecular weight heparin and aspirin in placental explants have been reported to depend upon the in vitro O2 concentration (Kleppa et al., 2014). These observations indicate the possibility that studies carried out using atmospheric O2 may not accurately reflect the in vivo environment.

Third, it has been repeatedly proposed that circulating maternal aPL inhibit trophoblast invasion of the maternal decidua, and this is one of the conclusions reached by Tong et al. (2015). However, there appears to be a temporal as well as a spatial problem with this theory of circulating aPL-associated early pregnancy loss. It has been shown that significant contact with maternal blood flow does not occur until the end of the first trimester (Burton et al., 2007, 2010). This raises an interesting question: could there be interference in trophoblast invasion by aPL in maternal blood before exposure to the circulating aPL has occurred? Further, if there is no contact with maternal circulation until the end of the first trimester, perhaps human serum is not the most appropriate source for aPL in some of these in vitro studies of early placental dysfunction. It would be of interest to determine if aPL are present in uterine glandular secretions (that bathe the first trimester trophoblast) (Filant and Spencer, 2014) in patients with antiphospholipid syndrome.

Lastly, with regard to the tissues used in studies reviewed by Tong et al., we believe the gender of the cells should be reported. Shah et al. (2014) recently argued that the sex of cells being used in experiments can impact the cell’s biology. The gender of cell lines is relatively simple to ascertain (for example, http://genome-mirror.duhs.duke.edu/ENCODE/cellTypes.html): JEG-3 and BeWo cell lines are both male, whereas HTR8swn is a female cell line. Considered in the context of findings regarding the predisposition of the male fetus to abnormal placental development (Murji et al., 2012), it would appear that the gender of the fetus from which a placental explant is obtained as well as the gender of a trophoblast cell line may influence findings, and should therefore be reported in studies of putative aPL-related damage.

The authors of this systematic review acknowledged that heterogeneity of available studies limited their ability to draw conclusions regarding aPL-mediated abnormal placental development. We think the limitations of the in vitro studies reviewed may be even more significant: (i) diverse and possibly unrepresentative models of early placentation; (ii) non-physiological culture conditions; (iii) the source of aPL; and (iv) the apparent lack of interaction between maternal circulation and trophoblasts in the first trimester. Tong et al. (2015) suggest much work remains to be done and we concur. We propose the next generation of studies might benefit from integration of new evidence regarding in vitro culture if we are to expand our understanding of if, how, and when aPL contribute to placental dysfunction.

References

Reply: In vitro culture conditions, antiphospholipid antibodies and trophoblast function

Sir,  
We thank Drs Clark and Laskin for their interest in our recently published systematic review of the effects of antiphospholipid antibodies on cultured placental cells in vitro. We agree with many of the authors’ comments and their suggestions for future investigations. However, in suggesting that trophoblast plugs prevent antiphospholipid antibodies accessing the placenta in early gestation, we believe that Drs Clark and Laskin have misinterpreted the reports of trophoblast plugs in the uterine spiral arteries.  
Firstly, it has been shown that potentially as few as 20% of spiral arteries are fully plugged during the first trimester of human pregnancy (Meekins et al., 1997), which if true, means maternal blood-borne antibodies would readily access early gestation placentae. Secondly, trophoblast plugs in the spiral arteries are widely agreed to be only loosely cohesive (Boyd and Hamilton, 1970; Ramsey and Donner, 1980). This loose cohesion means that while the plugs prevent the passage of maternal red blood cells into the intervillous space, maternal plasma may still pass through the plugs to access the placenta. Supporting this, hysteroscopic examination reveals that placental villi are bathed in a clear fluid prior to 12 weeks of gestation (Jaffe et al., 1997) and histologic specimens of implantation sites from as early as 50 days gestation show clear tracks of fluid leading from the spiral arteries to the intervillous space (Burton et al., 1999). The flow of maternal plasma to and from the placenta is corroborated by the finding of substantial numbers of placenta-derived extracellular vesicles (syncytiotrophoblastic microvesicles) in the maternal peripheral blood from as early as 6 weeks of gestation (Covone et al., 1984; Knight et al., 1998; Askelund and Chamley, 2011; Salomon et al., 2014). Thus, unlike maternal red blood cells, soluble factors including antiphospholipid antibodies have access to the first trimester placenta despite the presence of trophoblast plugs in the spiral arteries. Therefore, these autoantibodies may have direct deleterious effects on the placenta from the beginning of placentation development.

B cell responses in pregnancy and vaccine efficacy

Sir,  
We read with great interest the review by Faucette et al. (2015) regarding the safety and impact of maternal immunization during pregnancy. This review is an exhaustive work encompassing the epidemiological, physiological and practical aspects of this critical global health issue. They argue that significant alteration of the humoral response occurs during pregnancy that could hamper vaccine efficacy. They propose that estrogens and pregnancy hormones have significant impact on B cells development and function. However, most of the evidence comes from murine studies or in vitro models. In humans, quantitative changes in the B cell compartment during pregnancy have indeed been described, although these alterations seem rather limited. The viral immunity and pregnancy (VIP) study that prospectively assessed the immune parameters of 50 women during pregnancy and in the post-partum period only showed a moderate but significant decrease in the absolute B cells numbers during the third trimester (Kraus et al., 2012). However, these quantitative changes do not seem to alter B cell responses during pregnancy. Indeed, both immunological and vaccine studies indicate that efficient memory and plasma cells responses can be induced during pregnancy. We have studied the proportion of B cell subsets in pregnant women with and without cytomegalovirus (CMV) infection. While pregnant women with primary CMV infection had prolonged expansion of activated and atypical memory B cells, no change in the proportion of peripheral blood B cells subsets was observed between healthy pregnant women during the first trimester, second trimester and immediate post-partum period and non-pregnant women (Dauby et al., 2014). The VIP

References