Monoclonal Antibody against Phosphatidylserine Inhibits InVitro Human Trophoblastic Hormone Production and Invasion.

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Naturally occurring antiphospholipid antibodies (aPLs) against cardiolipin- and phosphatidylserine-dependent antigens are associated with placental dysfunction and unsuccessful pregnancy. Recently, we have reported that murine monoclonal aPLs react with human placental trophoblast and may interfere with normal trophoblastic function. In this study, we evaluated the expression of phospholipid-dependent antigens during trophoblast differentiation and measured the effects of monoclonal aPLs on two in vitro aspects of trophoblast differentiation, hormone production and invasion into filters coated with extracellular matrix.

The murine monoclonal IgM aPLs that differentiated between PS and CL were used; 3SB9b reacted only with PS (CL-/PS+), D11A4 reacted with CL (CL+/PS-), BA3B5C4 reacted with both CL and PS (CL+/PS+). Isolated trophoblast were cultured for four days, and reactivity with monoclonal aPLs was evaluated daily. BA3B5C4 (CL+/PS+) reacted strongly with most trophoblast that were freshly isolated (day 0) and through 2 days of culture, after which time the percentage of cells reactive with BA3B5C4 decreased steadily. 3SB9b (CL-/PS+) reactivity increased during incubation; very few cells reacted initially, but after 1 day of incubation 100% reacted, and this percentage remained stable throughout the 4 day incubation. D11A4 (CL+/PS-) reacted only minimally and at the level of the negative control monoclonal antibody with 1 and 2 day cultures. In terms of hormone secretion, by day 3 of culture, BA3B5C4 and 3SB9b had significantly inhibited basal hCG and hPL secretion (p<0.0005, p<0.0003, respectively). BA3B5C4 had reduced hCG and hPL secretion to 55% and 42% of the controls, and 3SB9b had reduced hCG and hPL secretion to 40% and 34% of the control levels, respectively. In contrast D11A4 did not inhibit basal hCG or hPL secretion. And we also investigated whether monoclonal aPLs would affect the cytotrophoblast invasion process in an in vitro model system using Matrigel-coated filters. First trimester trophoblast exposed to the PS-reactive aPLs, BA3B5C4 and 3SB9b, were completely blocked from traversing the filters. No cells were detectable on the filter after 72 hours of culture with either aPL. In cultures exposed to D11A4 or negative control (ds-DNA), 33.5 ± 4.0 and 35.0 ± 8.5 (cells/membrane) were observed to have traversed the membrane. Both monoclonals that reacted with PS-dependent antigens completely prevented invasion of matrigel coated filters by isolated
trophoblast.

Our results have suggested that throughout pregnancy the trophoblastic cells express multiple antigenic forms of PS-dependent antigens. Only monoclonal aPLs with specificity against PS-dependent epitopes react with trophoblast, and at least two different PS-dependent epitopes are expressed in the placenta. The differential reactivity of 3SB9b and BA3B5C4 suggests that the antigenic conformation involving PS on the cytotrophoblast is modulated into a different conformation concurrent with fusion into the syncytium. Trophoblast differentiation can be measured by the production of hormones, such as hCG and hPL. Our data demonstrate that aPLs against PS-dependent antigens will prevent the secretion of hCG and hPL. The process by which this occurs is yet unknown. Hormone production appears responsive to signal transduction through membrane bound phospholipase C and Protein kinase C. Sera containing aPLs can effectively block the induction of hCG production by exogenous phospholipase C or by gonadotropin-releasing hormone. The activity of protein kinase C is dependent on membrane phospholipid, particularly PS. It is possible, therefore, that aPLs against PS can interfere with signal transduction in trophoblast and prevent the induction of hormone production by the syncytiotrophoblast. Successful pregnancy is also dependent on extravillous trophoblastic invasion of the decidua. Proliferating villous cytotrophoblasts penetrate the overlying syncytium at the tip of some chorionic villi to form anchoring cell columns connected to the decidua. Trophoblasts migrate from these regions into the maternal tissue, some to the level of the maternal myometrium. Endovascular cytotrophoblasts, arising from the invading trophoblasts, extend into the maternal spiral arteries, displacing the endothelial cell lining of the maternal vessels. Our data demonstrate, for the first time, that aPS will prevent trophoblastic invasion in an in vitro model. Again, the mechanism has yet to be clarified. In vitro invasion is dependent upon several factors, including adherence to the extracellular matrix, response to external cytokine signals, expression and alteration of adhesion proteins, and production and secretion of proteases. The effects of aPLs on the individual steps in this process have not yet been studies.