OBSTETRICS

Human trophoblasts confer resistance to viruses implicated in perinatal infection

Avraham Bayer, BSc; Elizabeth Delorme-Axford, PhD; Christie Sleigher, MS; Teryl K. Frey, PhD; Derek W. Trobaugh, PhD; William B. Klimstra, PhD; Lori A. Emert-Sedlak, PhD; Thomas E. Smithgall, PhD; Paul R. Kinchington, PhD; Stephen Vadia, PhD; Stephanie Seveau, PhD; Jon P. Boyle, PhD; Carolyn B. Coyne, PhD; Yoel Sadovsky, MD

OBJECTIVE: Primary human trophoblasts were previously shown to be resistant to viral infection, and able to confer this resistance to nontrophoblast cells. Can trophoblasts protect nontrophoblastic cells from infection by viruses or other intracellular pathogens that are implicated in perinatal infection?

STUDY DESIGN: Isolated primary term human trophoblasts were cultured for 48-72 hours. Diverse nonplacental human cell lines (U2OS, human foreskin fibroblast, TZM-bl, MeWo, and Caco-2) were preexposed to either trophoblast conditioned medium, nonconditioned medium, or miR-517-3p for 24 hours. Cells were infected with several viral and nonviral pathogens known to be associated with perinatal infections. Cellular infection was defined and quantified by plaque assays, luciferase assays, microscopy, and/or colonization assays. Differences in infection were assessed by Student *t* test or analysis of variance with Bonferroni correction.

RESULTS: Infection by rubella and other togaviruses, human immunodeficiency virus-1, and varicella zoster was attenuated in cells preexposed to trophoblast-conditioned medium ($P < .05$), and a partial effect by the chromosome 19 microRNA miR-517-3p on specific pathogens. The conditioned medium had no effect on infection by *Toxoplasma gondii* or *Listeria monocytogenes*.

CONCLUSION: Our findings indicate that medium conditioned by primary human trophoblasts attenuates viral infection in nontrophoblastic cells. Our data point to a trophoblast-specific antiviral effect that may be exploited therapeutically.

Key words: chromosome 19 microRNA cluster, conditioned medium, microRNA, trophoblasts, viruses

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Beyond providing a physical barrier between the maternal and fetal vasculature, the placenta governs the exchange of gases, nutrients, and waste products between these 2 compartments. In the hemochorial placenta, this exchange is regulated primarily by the syncytiotrophoblasts, a layer of multinucleated, terminally differentiated cells that are bathed in the maternal blood and play a critical role in protecting the developing fetus from invading pathogens.[1] Despite this defensive barrier, some pathogens are able to invade the fetal environment.

Viral infection of the intrauterine compartment can spread to the fetus and/or the mother. Active maternal viral infections can lead to infection during delivery or to pregnancy loss (either early or late) resulting from systemic spread of the infection.[2] Viruses that are

From the Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Research Institute (Mr Bayer and Dr Sadovsky); Center for Vaccine Research (Drs Trobaugh and Klimstra) and Drug Discovery Institute (Dr Smithgall), Department of Microbiology and Molecular Genetics (Drs Delorme-Axford, Emert-Sedlak, Smithgall, Coyne, Sadovsky, and Kinchington); and Department of Ophthalmology (Dr Kinchington), University of Pittsburgh School of Medicine, Pittsburgh, PA; Department of Biology, Georgia State University, Atlanta, GA (Ms Sleigher and Dr Frey); Center for Microbial Interface Biology and Department of Microbial Infection and Immunity, Ohio State University, Columbus, OH (Drs Vadia and Seveau); and the Department of Biological Sciences, Dietrich School of Arts and Sciences, University of Pittsburgh, Pittsburgh, PA (Dr Boyle). Drs Coyne and Sadovsky contributed equally to this work.

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C.B.C. and Y.S. are named inventors on a pending patent application describing the use of chromosome 19 microRNA clusters as therapeutics.

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Corresponding author: Yoel Sadovsky, MD. ysadovsky@mwri.magee.edu

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transmitted directly to the fetus can result in developmental abnormalities or fetal or neonatal disease. For example, prior to the widespread use of vaccination, fetal infection rates by the rubella virus were nearly 50% during maternal rubella infection in the first trimester of pregnancy and were associated with congenital rubella syndrome, characterized by deafness, cataracts, damage to the central nervous system, and cardiac defects.[3] Congenital infection with varicella during pregnancy can lead to spontaneous abortion or neonatal varicella infection, which may result in devastating birth defects known as congenital varicella syndrome.[4,5] Venezuelan equine encephalitis virus (VEEV) has been linked to pregnancy complications such as spontaneous abortion.[6] Human immunodeficiency virus (HIV-1) tends to be transmitted during vaginal delivery or invasive procedures.[7]

Beyond the formation of a syncytial physical barrier, mechanisms by which placental trophoblasts influence viral infections are insufficiently understood. We recently demonstrated that primary human trophoblasts (PHT) are resistant to infection by an unrelated panel of viruses.[8] Furthermore, viral resistance was conferred to nontrophoblast cells when incubated with conditioned medium from PHT cells. This resistance was mediated, at least in part, by exosomal delivery of microRNAs (miRNAs) from the chromosome 19 miRNA cluster (C19MC), which is the largest miRNA cluster in human beings unique to primates and almost exclusively expressed in the placenta.[9] C19MC miRNAs are highly expressed in exosomes released from PHT cells, and can be found circulating in the plasma of pregnant women.[10,11] In cells exposed to PHT-conditioned medium we also observed a strong induction of autophagy, a pro-survival catabolic process where cellular organelles are partly or fully enclosed in cytoplasmic phagosomes, and degraded upon fusion with the lysosomes. Autophagy was also observed in cells transfected with selected miRNA members of the C19MC, and attenuation of autophagy mitigated this antiviral effect.[8] Here we expand upon our previous observations and focus on viruses that are pertinent to fetal infection during pregnancy and/or delivery, including rubella virus and other togaviruses, HIV-1, and varicella zoster virus (VZV). Additionally, we compared these effects to infection by 2 clinically relevant non-viral perinatal pathogens, *Toxoplasma gondii* and *Listeria monocytogenes*.

Materials and Methods

Study design

To assess the ability of human trophoblasts to confer pathogen resistance to nontrophoblastic cells, we collected conditioned medium from PHT cells, cultured from 48-72 hours, or control nonconditioned medium. This medium was added to nontrophoblast recipient cells for 24 hours prior to infection. Pathogens were then added to cells exposed to either medium, and subsequent infection was assessed utilizing the assays listed below. Infection was quantified relative to control conditions. This design is illustrated in Figure 1.

Cells

Human osteosarcoma U2OS, human foreskin fibroblast (HFF), melanoma-derived cells (MeWo), and TZM-bl cells[12] were cultured in Dulbecco modified Eagle medium (DMEM; Corning, Manassas, VA) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO) and antibiotics. Human epithelial cells (Caco-2 cells, ATCC [Manassas, VA] HTB-37) were cultured in Eagle minimal essential medium (MEM) containing 20% heat-inactivated FBS and 10 U penicillin and streptomycin. Vero African green monkey kidney cell line, commonly used for assessment of viral infections, were maintained in DMEM supplemented with 5% FBS and antibiotics.

PHT cells were acquired from healthy singleton term placentas, using the procedure previously described,[13] with modifications.[14,15] Cells were maintained in DMEM containing 10% bovine growth serum (HyClone, Logan, UT), 20 mmol/L HEPES, and antibiotics at 37°C. Cells were maintained 72 hours after plating, with cell quality monitored morphologically and by human chorionic gonadotropin levels (enzyme-linked immunosorbent assay; DRG International, Mountainside, NJ) in the medium, which show a characteristic increase as cytotrophoblasts differentiate into syncytiotrophoblasts.[16]

Conditioned medium preparation

Conditioned medium samples were collected from PHT cultures as previously described, and only medium that demonstrated at least 70% reduction in vesicular stomatitis virus (VSV) infection was used for subsequent infectivity assays.[8] Briefly, U2OS cells were exposed to conditioned or nonconditioned PHT medium for 24 hours, and infected with VSV at a multiplicity of infection (MOI) of 1 for 6 hours or until cytopathic effect was evident. Cells were then lysed with 1 mL of Qiazol lysis reagent (Qiagen Sciences, Germantown, MD), and infection was quantified by reverse transcription quantitative polymerase chain reaction, as previously described.[8] Notably, 65% of the media screened exhibited at least 70% reduction in VSV infection, and thus used for subsequent assays.

Rubella plaque assays

Vero cells were preexposed to either conditioned or nonconditioned medium and infected with rubella virus at an MOI of 10. Plaque assays were performed with serial dilution of the virus. A total of 1 mL of each dilution and a control (phosphate buffered saline [PBS]/1% FBS) were plated on 30-mm plates confluent with Vero cells, and the plates were incubated for 1 hour at 37°C. Cells were overlaid with a liquid agar solution (60 mL 0.4% liquid agar, 34 mL 3X MEM, 1 mL FBS, 3 mL 5% $NaHCO_3$, 0.1 mL penicillin/streptomycin, and 0.1 mL diethylaminoethanol). The plates were incubated for 7 days at 37°C. After incubation, the agar was removed, and the plates were stained with crystal violet solution to reveal plaques. Duplicate plaque assays were performed for each infection, and the final titer of each infection was the average of the 2 plaque assays.

Alphavirus luciferase assays

Vero cells were preexposed to either conditioned or nonconditioned medium