Bacterial membrane vesicles can cause preterm birth

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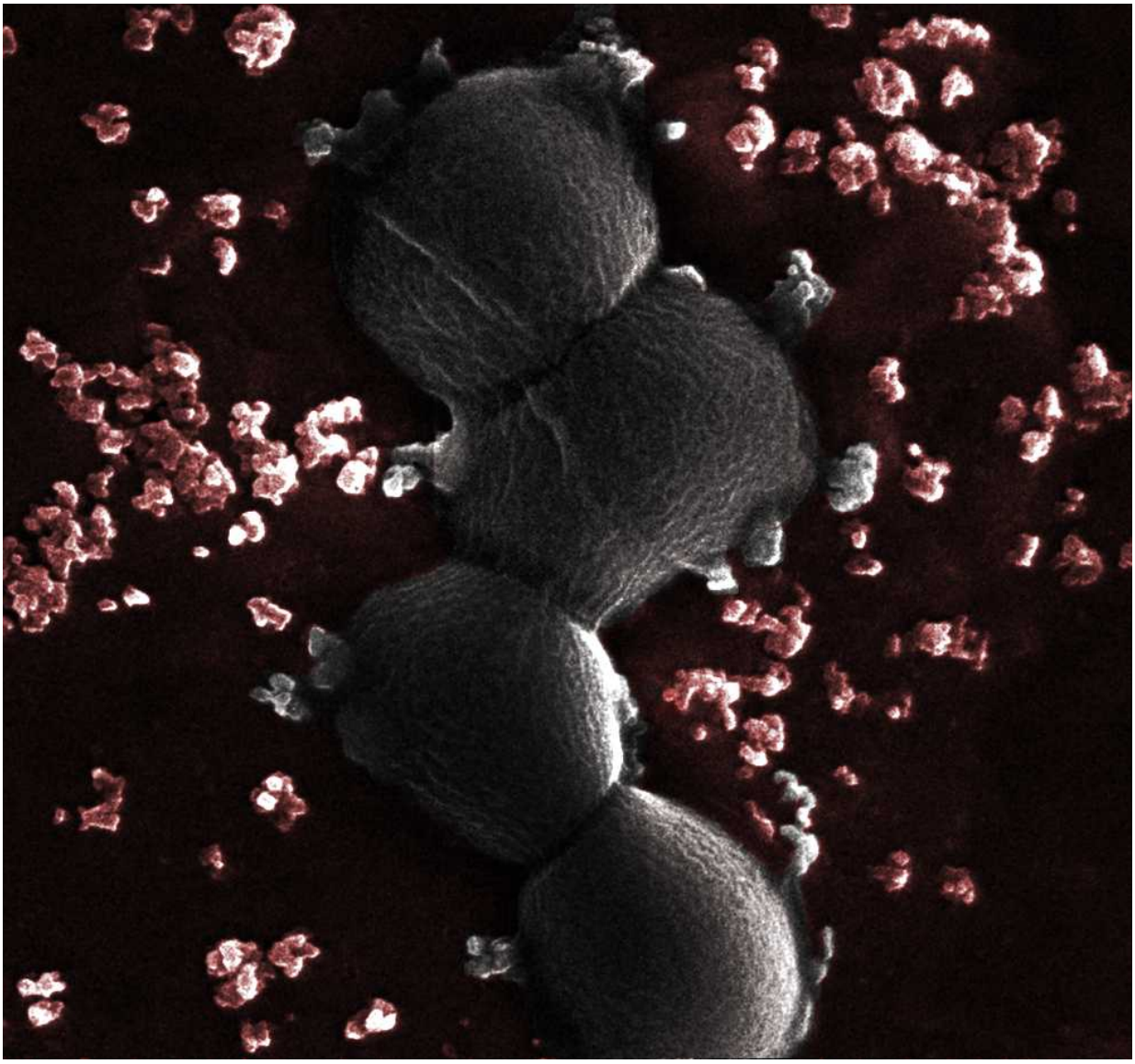

As a novel mechanism contributing to streptococcal pathogenesis, Surve et al.

identified membrane bound vesicles (MVs) produced by *Streptococcus agalactiae*. The scanning electron micrograph depicts MVs (red hue) dispersed around *S. agalactiae* cells (gray). See Anirban Banerjee and colleagues. Credit: Anirban Banerjee and colleagues

Approximately 20-30% of women carry bacteria called group B streptococcus (GBS) in their vagina or rectum. In most cases, these bacteria cause no problems, but GBS has been linked to complications during pregnancy, including pre-term delivery.

A study published on September 1st in *PLOS Pathogens* reports that GBS produces membrane-bound vesicles containing bacterial factors that can attack the host tissue. In mice, the study shows, these vesicles can move from the vagina to the uterus and cause inflammation of the membranes surrounding the fetus. When injected directly into the amniotic cavity of mice, these vesicles can induce preterm and still births.

Membrane-bound vesicles (MVs) loaded with toxins, immune-modulators, and other bacterial factors, contribute to the survival and virulence (the ability to cause disease) of many pathogenic bacteria. Whether GBS produces MVs was not known. However, because in pregnant women who carry GBS and deliver prematurely, bacterial infection is rarely found in the womb, Anirban Banarjee, from the Indian Institute of Technology Bombay, and colleagues hypothesized that if GBS produces MVs, they might move up to the womb during pregnancy and cause tissue damage at interface between mother and fetus.

To test this, the researchers started by growing GBS in liquid media. When they removed the bacteria and examined the remaining liquid by electron microscopy, they found numerous spherical structures. Zooming

in on the surface of growing bacteria, they detected vesicles that were just budding off the bacterial cell, confirming that GBS produces MVs. They next examined the protein content of the MVs and identified 8 bacterial proteins, all with predicted properties of virulence factors that can attack the human host and cause disease.

When the researchers mixed MVs and cells of human origin, they found that the MVs can invade and kill these cells, suggesting that GBS MVs are toxic to the human host. The researchers then deposited the MVs without the bacteria into mouse vagina and hours later found them throughout the uterus and in the developing fetus, indicating that MVs can indeed travel up the birth canal. Adding MVs to mouse chorio-decidual membrane (which is found at the interface between mother and fetus) caused collagen degradation, reducing the elasticity and weakening the mechanical properties of the membrane.

When the researchers injected MVs directly into the amniotic sac (the fluid-filled cavity surrounding the embryo) of pregnant mice, they observed that 24 hours later the tissue of the interface between mother and fetus was severely disrupted, with broken collagen fibers, hallmarks of inflammation, and signs of extensive cell death. To test whether these changes could lead to pre-term birth, the researchers carefully monitored females whose amniotic sacs had been injected with MVs at day 14.5 of pregnancy (a full-term mouse pregnancy lasts 19 days). Approximately 60% of the fetuses were born prematurely (by day 18 of pregnancy), compared with only 10% of pups following control injection with saline. Along with preterm birth, the researchers observed an increased frequency of fetal death in utero, and that the pups born to MV injected mothers were too small and some had abnormal morphology. Collectively, these results suggest that GBS MVs can cause preterm birth and fetal injury.

Discussing their results, the researchers emphasize the finding that MVs

alone could induce features resembling clinical chorio-amnionitis in the mice. "Clinically", they say, "this observation is highly relevant as 50-80% women with chorio-amnionitis do not have bacteria in their amniotic fluid or the decidual tissue". Based on their study, they hypothesize that "MVs secreted by the pathogens residing in lower genital tract may be responsible for cases with unexplained chorio-amnionitis".

Acknowledging the gap between experimental results in mice following direct injection of MVs into the amnion sac and human pathogenesis, the researchers nevertheless suggest that their findings "provide a novel insight into how GBS while simply sitting in the vagina can orchestrate events at the fetal membrane leading to premature birth". Because MVS are not susceptible to antibiotics, the researchers speculate that instead "new drugs that prevent vesicle production may [...] be a viable therapeutic option to prevent GBS mediated preterm birth".

More information: Surve MV, Anil A, Kamath KG, Bhutda S, Sthanam LK, Pradhan A, et al. (2016) Membrane Vesicles of Group B Streptococcus Disrupt Feto-Maternal Barrier Leading to Preterm Birth. *PLoS Pathog* 12(9): e1005816. DOI: 10.1371/journal.ppat.1005816