Novel Replication Profiles of *Brucella* in Human Trophoblasts Give Insights Into the Pathogenesis of Infectious Abortion

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(See the major article by Salcedo et al on pages 1075–83.)

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Many infectious agents cause abortion in humans and in animals. In this issue of the *Journal of Infectious Diseases*, an article by Salcedo et al [1] describes the behavior of *Brucella* strains in human trophoblasts and presents data that change the current paradigm regarding *Brucella* virulence. Brucellosis is a serious disease caused by bacteria of the genus *Brucella*. The disease affects all species of farm animals, although it is most important when it affects ruminants. Brucellosis has a worldwide impact in terms of its epidemiology, human health risks, and effects on trade. In most natural animal hosts the predominant symptom is abortion, with consequent loss of offspring and milk yield. In males, orchitis and epididymitis occur with a resulting loss in fertility. Three species, *B. melitensis*, *B. abortus*, and *B. suis*, can be readily transmitted to man, either following professional contact with infected animals or following the ingestion of contaminated dairy products. Despite much effort worldwide, no vaccine is available for human prophylaxis, but infections can be treated with a combination of antibiotics.

*Brucella* is a facultative intracellular pathogen that can survive and replicate in many types of host cells, with macrophages as prime targets. This ability of *Brucella* to replicate intracellularly is central to its pathogenicity. When *Brucella* infects pregnant animals, it colonizes the trophoblasts in the placenta where it grows to very high density. In the mid-1980s, seminal studies from the Cheville lab showed that during placentitis of goats, *Brucella* were first seen in phagosomes in erythrophagocytic trophoblasts and in a compartment resembling rough endoplasmic reticulum in chorioallantoic trophoblasts [2, 3]. Although brucellosis is recognized as a cause of infectious abortion in animals, evidence that it causes abortion in humans is less clear.

In the late 1990s, studies expanded on the observations in goat placentas, unraveling the cell biology of *Brucella* infections using HeLa cells [4, 5]. Unlike certain intracellular pathogens that escape from the phagosome and multiply freely in the cytoplasm, *Brucella* stays within a membrane bound *Brucella*-containing vacuole (BCV). The Gorvel group used confocal microscopy to follow the interactions of the BCV with the endocytic pathways in the cell, finding that it transiently interacts with early endosomes, late endosomes and lysosomes. In these early stages, BCVs are positive for the lysosomal membrane-associated protein 1 (LAMP1). Acidification of the BCV is essential to induce expression of genes encoding virulence factors, including the VirB type IV secretion system. *Brucella* then replicates in a novel compartment built by capturing vesicles derived from the endoplasmic reticulum (ER). This compartment has been seen in both phagocytes and nonprofessional phagocytes in vitro [6, 7] and in trophoblasts of infected animals [21–23].

The placenta has many roles; it allows the passage of nutrients and waste products between the maternal and fetal blood steams, acts as an immunological barrier providing tolerance to the fetal allograft, and acts as a barrier to prevent transmission of infectious agents from the mother to the fetus. The placenta is, in fact, composed of both maternal and fetal cells. Four days after fertilization, the early stage embryo enters the uterus and develops into a blastocyst. The outer layer of the blastocyst (cytotrophoblasts)
implants into the uterine wall and starts to form the placenta. The cytotrophoblasts can differentiate into different cell types, including syncytiotrophoblasts, which have roles in transport between mother and fetus and also secrete hormones, and extravillous trophoblasts (EVTs), which are invasive and can penetrate the decidua to anchor the placenta. Erythritol, a 4-carbon sugar alcohol is the preferred carbon source for Brucella [8]. Erythritol is found in ruminant placentas, and its absence has been suggested as the reason that abortion is not a common symptom in human brucellosis. There have been some reports in which bacteria were isolated from fetal or placental tissue [10, 11], but there have been no reports describing the interaction between Brucella and human trophoblasts. The article by Salcedo et al in this issue starts to fill this gap in our knowledge on this topic.

Salcedo et al [1] infected the human trophoblastic cell line JEG-3 with virulent Brucella. This cell line has many features in common with EVT. In JEG-3 cells, B. abortus and B. suis replicate well. However, unlike with other cell types tested, replication is not completely dependent on the VirB T4SS, because a B. abortus virB mutant is still able to replicate to some extent. Strikingly, both B. abortus and B. suis replicated in large, LAMP-1 positive compartments in EVT. ER derived BCV. Although established cell lines offer a convenient model, they also differ from trophoblasts found in the placenta. In the current study, the authors isolated trophoblasts from term placentas. Interestingly, 40% of the preparations were resistant to Brucella infection, and were able to eliminate the bacteria. In preparations from other donors, B. melitensis and B. abortus could replicate to high numbers in a VirB-dependent manner. The observation that primary trophoblasts from certain donors were resistant to Brucella infection suggests genetic resistance. Little is known about the genetic basis of resistance to brucellosis; in cows and buffalos there have been suggestions that polymorphisms in the 3′ UTR of SLC11A1 and in the gene encoding the Nramp1 protein can modulate resistance [14–17], whereas interleukin-17A polymorphisms have been reported to influence human brucellosis [18].

In a very minor population of cells, Salcedo et al [1] also observed B. abortus but not B. melitensis, growing in inclusions. When the rare EVTs present in term placentas, enriched by plating on fibronectin-coated surfaces, were infected, inclusions were seen with B. abortus and B. suis, but not B. melitensis, in a similar fashion to the EVT-like JEG-3 line. As EVT also appear to be able to control B. abortus and B. suis, this finding suggests that these cells provide a barrier to the spread of bacteria to the remaining placenta and the fetus in humans. The authors note that Listeria infection has impaired virulence in human EVT cells, where it is unable to escape the phagosome to multiply in the cytoplasm [19, 20]. B. melitensis is clearly more virulent in EVT than B. abortus and B. suis, as it is able to reach its preferential ER derived niche. The 3 Brucella species used in this study cause the most serious infections in humans. At the genetic level, these 3 species are highly related, with most genes showing almost 100% identity at the DNA level, and there are only a limited number of genes or regions missing in one or more of the 3 species [21]. It will be interesting to determine whether differences in virulence among these species are due to an additional virulence factor, a virulence factor with a small polymorphism, or a difference in gene regulation.

The Salcedo et al study raises a wide range of new questions concerning the virulence of Brucella and its role in causing abortion in humans. However, the study does not address whether Brucella infection of trophoblasts occurs during infection in human pregnancy, and whether it is a cause of abortion. This question will require careful studies in areas of the world where brucellosis is still a problem. Unfortunately, the greatest impact of brucellosis is in the poorer, rural areas of the world, where access to medical and scientific infrastructure is often limited.

Notes

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