Abortive Potency of *Chlamydomphila abortus* in Pregnant Mice Is Not Directly Correlated with Placental and Fetal Colonization Levels

Amel Bouakane, Ilhem Benchaieb, and Annie Rodolakis*  
Unité de Recherche Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, Centre de Tours-Nouzilly, 37380 Nouzilly, France  

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* Corresponding author. Mailing address: Unité de Pathologie Infectieuse et Immunologie, INRA-Centre de Tours, 37380 Nouzilly, France. Phone: 33 2 47 42 78 88. Fax: 33 2 47 42 77 79. E-mail: annie.rodotakis@tours.inra.fr.

*Chlamydomphila abortus*, an obligate intracellular bacterium, colonizes different types of placenta and causes enzootic abortion during the last trimester of gestation in ewes and goats. The bacteria also present a potential zoonotic risk to pregnant women (6, 15, 23, 37).

This species is considered to be very homogenous at genomic (10, 21, 29) and antigenic levels (31). However, little genomic divergence has been identified (2, 3, 32), although variations between strains have been related to cross-protection in mouse models (14, 27). As far as we are aware, only three strains of *C. abortus*, i.e., LLC and POS, isolated in Greece from an aborted goat and ewe, respectively (32), and AB16, isolated in France from an aborted ewe in a vaccinated flock (18), vary in terms of reactivity with monoclonal antibodies against the highly immunogenic (9, 33) polymorphic outer membrane proteins (POMP) (17, 36). In addition, LLC and POS differ from other abortive strains in inclusion morphology and reactivity with protective monoclonal antibodies (11) against the major outer membrane protein (36). The numerous copies of genes encoding POMP (13, 16, 17, 19) suggest an essential role of POMP in the survival of chlamydiae (22), perhaps in the avoidance of host immune defenses (34). These three strains might therefore present differences in virulence, and previous studies (32, 36) have reported that strain LLG was more infectious for chicken embryos and cell cultures than other strains.

Generally, virulence is defined by the capacity of a bacterium to infect and damage its host, although this definition does not fully take into account the importance of the colonization stage. In this study virulence was evaluated both by abortion and by the individual colonization of placentas and fetuses. In addition, in view of its harmful effects in placenta (12, 20, 24), the induction of gamma interferon (IFN-γ) by the different strains was investigated. Indeed IFN-γ and tumor necrosis factor cause inflammation and together are thought to threaten the maintenance of pregnancy.

To assess the virulence of the strains, 8-week-old female OF1 (Swiss IFFA Credo, l’Arbresle, France) mice were inoculated intraperitoneally at 11 days of gestation, estimated as described previously (1). As in natural infection in ewes, intraperitoneal inoculation in pregnant mice during mid-gestation resulted in placental colonization, causing abortion and bacterial shedding at the end of gestation (4). In the first experiment, 5 of 20 pregnant mice were sacrificed from each infected group. Placentas and fetuses were individually removed (1) and titrated. In the second experiment, the pooled placentas and fetuses from the uterine horns of 10 pregnant mice per group were titrated (25). Blood collected from 10 pregnant and 5 nonpregnant mice was used to evaluate IFN-γ induction.

The average number of living mouse pups per litter of the 15 remaining mice per group was significantly lower for all inoculated strains than those of the control group (P < 0.0001), but it varied according to the strain. Mice inoculated with AB7 and POS did not differ significantly, with an average of 2.7 live pups per mother 8 days after birth. No difference was observed between AB16 and LLG under the same conditions, with 5.5 and 6.6 live surviving mice per litter, respectively (P = 0.43). However, there was a significant difference between groups AB16/LLG and AB7/POS (P < 0.0001), and the average numbers of live pups corresponded to four categories of birth. Inoculation of strains AB16 and LLG resulted in more “reduced litters” than real abortions, but they also resulted in some “pathological” and even normal litters (Fig. 1). AB7 and POS strains were the most virulent, since they mainly resulted in abortion.

To examine whether abortion and reduced litters resulted from bacterial multiplication in placentas and fetuses, chlamydia were counted in these organs individually. All the placentas were infected irrespective of the strains inoculated but at different levels (Fig. 2). Strains AB16 and AB7 colonized the placenta and fetus more strongly than strains POS and LLG (P < 0.0001). Therefore, contrary to our hypothesis, abortions and reduced litters were not correlated with the level of placental and fetal infection. In utero-infected mouse pups survived after inoculation of AB16. Abortion was not directly related to the number of bacteria in the placenta, and it was probably not the lysis of infected placenta cells alone that was responsible for abortion. Other factors may play an important role in activating the abortion process. Inflammatory cytokines, such as IFN-γ, produced in response to infection at the ma...
Internal-fetal interface have been postulated to abrogate normal placentation and predispose the mother and fetus to adverse reproductive outcomes, including miscarriage and fetal growth restriction (7, 20). The role of IFN-γ in abortion in mice infected with C. abortus AB7 has been demonstrated in a knockout mouse model, since IFN-γ knockout mice aborted earlier than the wild-type mice in spite of a lower level of placental infection (I. Benchabeb, personal communication).

Infected mice produced IFN-γ for all strains, but different levels were recorded. Inoculation of the AB7 strain induced very high levels of IFN-γ in nonpregnant mice, significantly higher than for controls and the other strains (P < 0.05). There was no difference in the induction of IFN-γ by the AB16, POS, and LLG strains (Fig. 3).

The levels of placental infection after inoculation with AB7 were similar to those evaluated by titration of pooled placentas or fetuses from the same uterine horn after intravenous inoculation of mice (28). Levels of AB16 in our experiment were similar to those of the strain that colonized placentas and fetuses most (28). In contrast, POS and LLG, which are undoubtedly C. abortus strains, gave the same kinds of results as strains belonging to the Chlamydophila pecorum species. Compared to other C. abortus strains, POS and LLG formed very small plaques of lysis in cell cultures, as for most C. pecorum strains, smaller than those caused by AB7 and AB16. Thus, the

FIG. 1. Average number of mouse pups surviving at 8 days postbirth corresponded to four categories of birth. Fifteen mice per group were inoculated intraperitoneally with 4.3 × 10⁵ PFU of AB7, 6.1 × 10⁵ PFU of AB16, 5.5 × 10⁵ PFU of POS, or 7 × 10⁵ PFU of LLG. Twenty unchallenged mice were kept as the control group. The mice were placed in individual cages, and mouse pups were counted from birth until 8 days postbirth for pregnancy follow-up (26). According to our observations with 200 mice, the average number of living pups in a normal litter was 11.91 ± 0.21. When there were fewer than nine, the litter was considered “reduced.” Abortion was defined as no living pups at birth. However, when all the pups died before the eighth day, it was considered to be a “pathological litter.”

FIG. 2. Comparison of fetal and placental colonization after intraperitoneal inoculation of 5 mice at 11 days of pregnancy with 4.3 × 10⁵ PFU of AB7, 6.1 × 10⁵ PFU of AB16, 5.5 × 10⁵ PFU of POS, or 7 × 10⁵ PFU of LLG. Five days after challenge, placentas and fetuses from the same uterine horn were individually and aseptically removed (1). Organs were kept individually and homogenized in phosphate-buffered saline–DEAE dextran (0.01%), and titrated by PFU (25) and enumerating inclusion-forming units (IFU) on McCoy cells (4). The levels of placental colonization of mice inoculated with strains POS and LLG did not differ (4.05 and 4.60 log, respectively) (P = 0.13) (two-factor analysis of variance, strains-horn), but they were significantly less heavily infected than those of mice inoculated with AB7 (5.28 log) (P < 0.001) or AB16 (6.21 log) (P < 0.001) (two-factor analysis of variance Strains-Horn). Only two fetuses were infected with the POS strain, whereas the LLG strain also resulted in many unaffected fetuses, which may explain the reduced litters. The placental colonization level of LLG was greater than that of POS, but the difference was not statistically significant.
rates of multiplication of the strains in cells could be responsible for the differences in levels of placental and fetal colonization. The cycle of multiplication must be compared to demonstrate whether AB16 differs from other strains and whether their cycles are shorter. The differences in numbers of chlamydiae in placentas and fetuses could also be due to different levels of IFN-γ in the blood. Indeed, the influence of IFN-γ on the multiplication of chlamydiae has been clearly demonstrated (30), but the serum levels of IFN-γ in mice were not related to the number of Chlamydia bacteria in placentas and fetuses.

Hormones can influence whether cells differentiate into those producing Th1 or Th2 (8), suggesting that they have a contributory role in abortion (12, 35) by increasing production of IFN-γ (5). It would be interesting to verify whether the hormonal response is the same after inoculation of different strains of C. abortus.

In our mouse model, differences in strain virulence were observed in terms of abortive effect, placental and fetal colonization, and level of IFN-γ in the blood. These three criteria were not correlated. Interestingly, strain AB16, which multiplied most in placentas and fetuses, did not induce the greatest number of abortions or the highest levels of IFN-γ. It is not possible to extrapolate these results to ewes, since the placental anatomies of mice and ewes are different, and it would therefore be interesting to compare how the four strains multiply in ovine placentas. Before undertaking such a study, it is necessary to specify the roles of each of the different factors and the cytokines involved for mice. Such studies using the available tools for mice should identify the virulence mechanisms of Chlamydia. These differences in virulence between strains cannot be linked to the differences in the POMP, since POS and LLG, which are considered to be two homologous strains for pmp genes, both for inclusion morphology and for their reactivity with antibodies against POMP (17, 36), were not similarly abortive in mice.

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