Histological and Immunocytochemical Characterization of CoxIELLA burnetii-Associated Lesions in the Murine Uterus and Placenta

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The fetoplacental units and the postgravid uterus of BALB/cJ (H-2b) mice inoculated intraperitoneally with CoxIELLA burnetii (Nine Mile isolate, phase I) on day 6 of pregnancy were examined histologically and immunocytochemically at 1 to 160 days postinoculation. Clinically, abortions, stillbirths, and perinatal deaths were observed. Histological lesions in the placenta were characterized by severe necrosis of the decidua basalis and the labyrinth, fibrinoid degeneration of decidual vessels, and microthrombosis. Pyometra and endometritis at the sites of previous placental attachment, characterized by ulceration, central necrosis, and moderate cellular infiltration consisting of neutrophils and macrophages, were observed postpartum. Pups sacrificed at the age of 9 days exhibited interstitial pneumonia with few granulomas and granulomatous hepatitis and splenitis. Immunocytochemically, antigen-bearing cells were first detected in the decidua 9 days postconception, and single immunopositive cells were detected in the fetal placenta 4 days later. Thereafter, until abortion or parturition, abundant accumulation of C. burnetii antigen was observed in the maternal and fetal compartments of the placenta. Up to 28 days postinoculation, many immunopositive cells were demonstrated at the sites of previous placental attachment, whereas the adjacent endometrium contained only a few antigen-positive cells. C. burnetii antigen was demonstrated in decidual cells, trophoblasts, and macrophages and extracellularly within the sinuses of the labyrinth and in the uterine lumen but not in granulated metrial gland cells. Fetuses in utero and aborted, stillborn, or perinatally dying offspring were immunocytochemically negative for C. burnetii antigen; however, pups killed 9 days after birth showed lesion-associated positive immunoreaction in the lung, liver, and spleen. The present study shows that infection with C. burnetii during pregnancy results in uncontrolled growth of the organism in the murine uteroplacental unit and that associated lesions are characterized by necrosis of placental tissues, fibrinoid degeneration of decidual vessels, and microthrombosis.

CoxIELLA burnetii, the etiological agent of Q fever, is an obligate, gram-variable, intracellular pathogen of the family Rickettsiaceae (49). Infection with C. burnetii has been demonstrated in arthropods, numerous free-living and domestic animal species, and humans (1, 40). In general, natural infection of animals is described as unapparent or dormant, and organisms are not excreted until parturition occurs (1, 40). Humans are usually infected by inhaling dust from dried ruminant fetal fluids. In humans, infection can be asymptomatic or result in acute or chronic disease (4, 5, 40). Acute disease is characterized by a febrile illness which is often diagnosed clinically as influenza or atypical pneumonia (4, 37). Chronic infection in humans results in valvular endocarditis, osteomyelitis, and hepatitis (4, 5, 15).

C. burnetii has a marked affinity for mononuclear phagocytes, and organisms can be demonstrated readily in the spleen, liver, and bone marrow during the acute phase of infection (5, 6a, 22, 40). Furthermore, C. burnetii has been detected in the gravid uterus and placental tissue of many species, including mice, guinea pigs, cattle, goat, sheep, and humans (1, 24, 25, 28, 30, 40, 44). In addition, Q fever outbreaks in humans were associated with parturient carnivores (26, 35). In animals, as in humans, aerosol infection represents the main route of infection; however, in contrast to humans, hepatic, cardiac, and respiratory tract lesions are not observed in livestock animals. Although infection is often unapparent in sheep, cattle, and goats, there is increasing evidence that C. burnetii infection in these species is associated with abortion, stillbirth, and delivery of weak offspring (1). However, studies of naturally occurring ruminant coxiellosis are hampered by species-specific differences. Sheep, in contrast to goats and cattle, rarely become chronically infected with C. burnetii, and abortion during coxiellosis epizootics have been described for goat and sheep, whereas the pathogenic potential for cattle is still controversial (24, 25, 40). Furthermore, investigations of sporadic outbreaks of C. burnetii-associated abortion are frequently complicated by concurrent infections and by the latent and subclinical course of the disease. Clinical analysis of reports on human C. burnetii infection during pregnancy revealed that perinatal infection occurs, but no abortion risk for humans was demonstrated (25). Investigations of the abortifacient potential of C. burnetii are further complicated by the fact that the organism can be isolated from placental tissue and birth products of animals and humans following abortion and normal birth (24, 25, 36, 39, 40, 44, 46, 50).

Despite different placental interhemal barriers, which are syndesmochorial for ruminants, endotheliochorial for dogs and cats, and hemochorial for rodents and humans (3), C. burnetii shows a high tropism for the gravid uterus, indicating a species-independent affinity for placental tissue. So far, most reports have focused on the epidemiological aspects of abortion- or birth-associated shedding of the organism, whereas only a few studies have investigated the morphogenesis of C. burnetii-associated lesions in the placenta.
Therefore, the objectives of this study were to characterize the histological lesions in the placenta of mice infected on day 6 of pregnancy and to immunocytochemically identify sites infected with Coxiella.

**MATERIALS AND METHODS**

**Organism and cell culture.** The Nine Mile isolate of *C. burnetii*, phase I (originally, kindly provided by Dr. Rebecca, Bratislava, Czechoslovakia), was used for the present study. The propagation, purification (kindly performed by N. Schmeir), and in vivo virulence of this strain have been described previously (6, 6a). Briefly, *C. burnetii* was propagated after 10 passages in mice in the yolk sacs of embryonated chicken eggs, harvested, and purified by Renografin gradient centrifugation. Titration of the stock suspension was performed in Buffalo green monkey cells, and titers are expressed as inclusion-forming units as described before (41).

**Mice.** Female and adult male BALB/cJ (H-2b) mice, 8 to 12 weeks old, were used in this study. The animals were purchased from the Zentralinstitut für Versuchstiere, Hannover, Germany. They were housed at 25°C and were given commercial-laboratory food and water ad libitum. Mice were tested serologically and microbiologically for common mouse pathogens, including mouse hepatitis virus and Sendai virus. Primigravid pregnancies were obtained by caging one or two females with one adult male. The time of conception was determined by the presence of a vaginal plug and designated day 0 of pregnancy.

**Infection of mice and clinical studies.** Animals were inoculated on day 6 of pregnancy. Thirty-three pregnant mice, selected at random, received 3.85 × 10^6 inclusion-forming units of *C. burnetii* intraperitoneally in 400 μl of phosphate-buffered saline (PBS). Seventeen pregnant animals were mock-infected with 400 μl of PBS. Mice were examined daily for clinical signs of infection. Animals were weighed on days 0 and 10 of gestation and daily thereafter until day 22 postconception (p.c.), and when they were killed. Rates of abortion, stillbirth, and birth (number of litters with abortions, stillbirths, and normal births as a percentage of all litters), pregnancy rate (percentage of actually pregnant mice among all animals with a vaginal plug), number of live-born pups per dam, and number of offspring surviving for 9 days after birth were determined.

**Histopathology and immunocytochemistry.** At various intervals after inoculation or when the animals were moribund (days 1, 3, 5, 7, 9, 11, 12, 13, 14, 15, 20, 28, 48, 78, and 160), the animals were killed by inhalation of CO₂ and decapitation. Offspring born alive were sacrificed 9 days postpartum. At necropsy, the spleen and uterus with the fetoplacental units of the dam and various organs, including the liver, lung, spleen, and bones, of the pups were collected. The uterus was fixed with pins on cardboard. All organs were immersed in Bouin’s fluid, and mineralized tissues were decalcified in 10% (wt/vol) EDTA disodium salt dissolved in double-distilled water, pH 7.4, for 72 h at room temperature (6). For routine histology, tissues were dehydrated, embedded in paraffin at 53°C, and stained with hematoxylin and eosin.

The methods used for immunocytochemical staining of *C. burnetii* and production of the rabbit anti-*C. burnetii* serum have been described previously (6). Briefly, 4-μm-thick tissue sections were mounted on gelatin-covered slides, dried, deparaffinized, and rehydrated. After quenching of the endogenous peroxidase, sections were sequentially incubated with rabbit anti-*C. burnetii* serum, biotinylated link antibody, and the avidin-biotin-peroxidase complex (ABC) (rabbit immunoglobulin G; Vectastain Kit; Vector Laboratories, Inc., Burlingame, Calif.). The polyclonal rabbit anti-*C. burnetii* antibody was used at a dilution of 1:3,000. A positive reaction was demonstrated by diaminobenzidine tetrahydrochloride (DAB)-H₂O₂ precipitation. Previous studies showed that blocking of endogenous blood was not necessary for tissues fixed in Bouin’s fluid (6). Control slides of tissues from *C. burnetii*-infected and uninfected mice were prepared. To confirm that staining was specific for *C. burnetii*, normal rabbit serum or PBS was used instead of primary and bridge antibody and the ABC reagent. Effective blocking of endogenous peroxidase and absence of reactive tissue blood were demonstrated by incubation of the sections with DAB and ABC. The intensity and distribution of the immunoreaction were scored subjectively as negative, minimal, moderate, or strong staining. The results for each organ are expressed, if not stated otherwise, as the mean score for all animals examined on the same day.

**Statistical analysis.** Differences between control and infected animals in survival of offspring to 9 days of age, occurrence of external vaginal bleeding, and pregnancy, abortion, stillbirth, and birth rates were determined by Fisher’s one-sided exact test. The two-tailed Wilcoxon rank sum test was applied to analyze the number of offspring born alive in each group. The statistical significance of the differences between groups in time to abortion, stillbirth, and normal birth was evaluated by the nonpaired Student’s t test.

To determine whether the difference in body weight between control and infected animals was significant, two-way analysis of variance with unbalanced repeated measures was performed. Results were regarded as significant when P was <0.05.

**RESULTS**

**Clinical signs and gross findings.** Infected animals showed lethargy, dehydration, and ruffled fur between 3 and 15 days after infection. One animal was found dead 12 days postinoculation (p.i.), and two were moribund at 9 and 14 days p.i. following abortion and stillbirth. In pregnant control mice, the body weight increased from 26.2 ± 1.5 g to 49.2 ± 6 g until parturition, compared with 34.2 ± 6.5 g for *C. burnetii*-infected animals at day 17 of pregnancy (Fig. 1). After gestation, body weights returned to prepregnancy values in both groups. Necropsy findings (fetoplacental unit) and clinical observations (increase in body weight and occurrence of abortion, stillbirth, and birth) for *C. burnetii*-infected animals revealed that 39 of 63 mice (62%) with a vaginal plug were pregnant, whereas in control animals the pregnancy rate was 82% (14 of 17). Eleven infected animals and seven control animals were necropsied before day 18 of pregnancy, and therefore, the possible reproductive failure of these animals could not be determined with certainty. *C. burnetii* infection-associated effects on reproductive performance and the significance of the findings for the remaining 28 infected and 7 control animals are summarized in Table I. Abortion, stillbirth, and normal birth were observed for 12, 14, and 2 infected pregnant mice, respectively. Abortion was not demonstrated until day 15 of pregnancy, and external vaginal bleeding, an indication of in utero fetal death or demise, occurred between days 13 and 18 of pregnancy. Among infected animals, 7 of 13 offspring born alive died within 24 h after birth, whereas 45 of 47 pups born to control animals were still alive at day 9 after birth.
In gravid females, gross findings such as hepatospleno-megaly and multifocal hepatic necrosis were prominent between 3 and 28 days after infection. Until day 9 of gestation, no macroscopic differences in the gravid uterus were observed between control and C. burnetii-infected animals. Thereafter, fetoplacental resorption and in utero fetal death, characterized by smaller embryo size, fetal necrosis, and placental hemorrhage, were demonstrated in all infected animals necropsied.

**Histological evaluation of the placenta and postgravid uterus in control mice.** The following terms are used for the different compartments of the gravid murine uterus (Fig. 2A) (23, 32). The innermost area of the fetal placenta, close to the embryo, represents the chorionic plate; this area is followed by the labyrinth with maternal sinuses and fetal capillaries, the trophospongium, and the giant-cell layer. The latter is close to the maternally derived decidua basalis, which is formed by hormonally modified endometrial cells. The decidua capsularis is found antimesometrially, and the lateral compartment is termed the decidua parietalis. The outermost region of the placenta, subjacent to the decidua basalis, represents the metrial gland, with granulated metrial gland (GMG) cells.

Control animals had no significant microscopic lesions and showed physiological degenerative changes of the fetoplacental unit, especially of the decidua basalis, between days 15 and 18 of pregnancy, as described by others (18, 32). Until 4 days postpartum, sites of previous placental attachment were characterized by focal necrosis, a few macrophages, neutrophils, and single persistent GMG cells. Focal accumulation of hemosiderin-laden macrophages was observed subjacent to areas of previous placental attachment until 84 days after conception.

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**Histological evaluation of spleen and uteroplacental units in infected mice.** Splenic lesions consisted of venous microthrombi in the early phase of infection. Between 3 and 48 days p.i., inflammatory changes were characterized by granulomas and pyogranulomas in the red pulp.

At days 9 and 11 of pregnancy (3 and 5 days p.i.), perivascular accumulations of neutrophils and macrophages were observed in the mesometrium and between the inner and outer layer of the myometrium in the stratum vasculare. Single vacuolated mononuclear cells were present in the decidua parietalis, decidua capsularis, and the metrial gland. At 13 days p.c. (7 days p.i.), infiltrating neutrophils were found in the decidua basalis, the metrial gland, and the endometrium. In addition, macrophages, erythrocytes, and cellular debris were observed in the uterine lumen. Between 15 and 20 days p.c. (9 and 14 days p.i.), severe diffuse necrosis and moderate cellular infiltration consisting of neutrophils and macrophages were present in the decidua basalis (Fig. 2B and C). In some animals, there was an area of necrosis without inflammatory cells subjacent to the giant-cell layer, followed by a second layer consisting of degenerated neutrophils and macrophages in the decidua basalis. In contrast, changes in the metrial gland were minimal, and only a few infiltrating neutrophils and macrophages were observed in addition. Fibrinous degeneration of maternal venules was a prominent finding in the decidua basalis (Fig. 2C). Furthermore, microthrombi were observed in decidual vessels.

The earliest histological lesions in the fetal placenta were demonstrated on day 13 of pregnancy (7 days p.i.). Changes especially prominent in the labyrinth were characterized by multifocal necrosis, mild neutrophilic infiltration, and vacuolation of trophoblasts. Between days 15 and 20 p.c. (9 and 14 days p.i.), inflammatory lesions and necrosis increased in severity. In addition, the labyrinth had a spongy appearance due to ectatic sinuses (Fig. 2B), and microthrombi were observed in the labyrinthine blood spaces (Fig. 2D). Numerous organisms, subsequently identified as coxiellae by immunocytochemistry, were demonstrated in the cytoplasmic vacuoles of trophoblasts in the giant-cell layer, trophospongium, and labyrinth. Furthermore, ectatic sinuses of the labyrinthine placenta were focally engorged with neutrophils and coxiella-laden macrophages. In addition, numerous extracellular coxiellae, forming emboli, were present in the maternal sinuses. Single-cell necrosis was also observed in the chorionic plate. In some animals, complete necrosis of the uteroplacental unit but not of the metrial gland was observed.

**Histological evaluation of the postgravid uterus in infected mice.** Until 26 days p.c. (20 days p.i.), following abortion and...
FIG. 2. Uteroplacental units of a control mouse and a *C. burnetii*-infected animal. (A) Uteroplacental unit of uninfected control animal. (B) Severe necrosis of decidua basalis and fetal placenta at 11 days p.i. Note ectatic sinuses in labyrinthine placenta. (C) Fibrinoid degeneration of decidua vessel (arrow) and severe necrosis of the surrounding decidua basalis with degenerated neutrophils and macrophages at 11 days p.i. (D) Microthrombus in dilated labyrinthine blood space and numerous *C. burnetii* organisms (arrow) at 14 days p.i. Abbreviations: C, chorionic plate; D, decidua basalis; F, fetal tissue; G, giant-cell layer; L, labyrinth; MG, metrial gland; T, microthrombus; Y, visceral layer of yolk sac. Hematoxylin and eosin stain. Bars: (A and B) 190 μm; (C) 28 μm; (D) 32 μm.
parturition, pyometra was a common finding in *C. burnetii*-infected animals. Lesions, prominent at sites of previous placental attachment, were characterized by superficial ulceration, central necrosis, and moderate cellular infiltration consisting of neutrophils, macrophages, and persistent GMG cells. Fibrin thrombi were occasionally demonstrated in small intralesional venules. The necrosis extended through the still disrupted inner layer of the myometrium into the stratum vasculare. Between 26 and 54 days p.c., sites of previous placental attachment were separated from the adjacent endometrium by several layers of fibroblast-like cells; the number of inflammatory cells declined, and GMG cells were no longer detectable. No significant microscopic lesions were observed on days 78 and 160 after infection.

Histological evaluation of fetuses and offspring. No inflammatory lesions were demonstrated in fetal tissues in utero or in aborted, stillborn, or perinatally dying offspring. All six pups killed at 9 days of age showed mild to moderate granulomatous hepatitis (Fig. 3A); granulomatous splenitis was also demonstrated in one offspring. Mild interstitial pneumonia with a few granulomas was observed in four animals (Fig. 3B). The remaining organs, including the alimentary tract, central nervous system, bone marrow, and kidneys, had no significant inflammatory lesions.

**Immunocytochemistry.** DAB precipitation products varied from coarse to fine grains and were found intra- and extracellularly. Intracellularly, *C. burnetii* antigen was found predominantly in cytoplasmic vacuoles. These inclusions were either packed with coarse granular dark brown immunopositive material or exhibited membrane-bound immunoreactivity. In some cells, *C. burnetii* antigen exhibited diffuse cytoplasmic distribution. Extracellularly, dark brown immunopositive material, coccobacillary in shape and 1 to 2 μm in length, presumably representing single organisms, was demonstrated. In areas of necrosis, *C. burnetii* antigen stained light brown and had a fine granular appearance, most likely representing residual particular antigenic debris.

*C. burnetii* antigen in spleen and uteroplacental units. The spread and distribution of *C. burnetii* antigen in the uterus and spleen are outlined in Table 2. In the spleen, numerous immunopositive cells were observed in the red pulp between 3 and 15 days p.i. (Fig. 4A). Thereafter, the number of immunopositive cells decreased, and few *C. burnetii* antigen-bearing cells were still present in one animal at day 78 after infection. *C. burnetii* antigen was observed in vacuolated phagocytes, granuloma-forming macrophages, and extracellularly.

At 9 days p.c. (3 days p.i.), few immunopositive cells were present in the decidua parietalis and decidua capsularis (Fig. 4B). Single *C. burnetii* antigen-bearing cells were found in the decidua basalis and the adjacent endometrium. Morphologically, immunopositive decidual cells varied from polygonal to spindle-shaped. At 11 days p.c. (5 days p.i.), the number of immunopositive cells in the decidua increased. Furthermore, at days 11 and 13 p.c., diffuse positive staining was demonstrated in areas with necrosis in the decidua capsularis and parietalis.

At 13 and 15 days p.c. (7 and 9 days p.i.), strong diffuse immunostaining was present in the decidua basalis, whereas only a few foci of immunoreactivity were demonstrated in the fetal placenta (Fig. 4C). Between 17 and 20 days p.c. (11 and 14 days p.i.), strong immunoreactivity was observed in the maternal and fetal compartments of the placenta (Fig. 4D). Immunostaining was especially prominent in the ectatic maternal vessels of the labyrinthine placenta. The labyrinthine blood spaces contained many immunopositive monocytes (Fig. 5A). In addition, extracellular positively staining coccobacillary organism-like material was found in the labyrinthine sinuses and the uterine lumen. In the fetal placenta, *C. burnetii* antigen was found in trophoblasts of the giant-cell layer, trophospongium, and labyrinth. However, unequivocal discrimination between immunopositive large cytoplasmic vacuoles and slightly dilated labyrinthine blood spaces was not always possible (Fig. 5B). Few immunopositive cells were observed in the endometrium, metrial gland, chorionic plate, visceral wall of the yolk sac, Reichert’s membrane, uterus epithelium cells, and uterine lumen. In these compartments, *C. burnetii* antigen was demonstrated in resident and granuloma-forming macrophages, epithelial cells, and extracellularly but not in GMG cells.

*C. burnetii* antigen in the postgravid uterus. Sites of previous placental attachment exhibited strong immunostaining postabortion and postpartum until 28 days after infection (Fig. 6). Only few immunopositive mononuclear cells were present in the other compartments of the uterus. At 28 and 48 days p.i., animals whose fetuses were aborted or stillborn were still immunocytochemically positive at the sites of previous placental attachment, whereas both animals who gave birth normally lacked *C. burnetii* antigen. At the sites of previous placental attachment, positive immunoreaction was

![Lesions in the liver (A) and lung (B) of a 9-day-old offspring from a *C. burnetii*-infected dam. (A) Single granuloma (arrow) in the liver and foci of extramedullary hematopoiesis (arrowhead). (B) Mild interstitial pneumonia and focal accumulation of macrophages and neutrophils. Hematoxylin and eosin stain. Bars, 35 μm.](http://iai.asm.org/)
restricted to macrophages and few fibroblast-like cells. No
C. burnetii antigen was demonstrated in GMG cells. In the
uterine lumen, positive immunostaining was observed until
15 days after infection. The DAB precipitation product was
present in macrophages and sloughed epithelial cells and
extracellularly.

C. burnetii antigen in fetuses and offspring. C. burnetii
antigen was not found in fetal tissues in utero or in aborted,
stillborn, or perinatally dying offspring. In contrast, positive
staining was observed in the liver, lung, and spleen of six,
four, and one offspring, respectively, at 9 days of age. In
these organs, C. burnetii antigen was present in migrating or
granuloma-forming macrophages. The remaining organs, in-
cluding the bone marrow, kidney, and central nervous
system, were immunocytochemically negative for C. burn-
etii antigen.

DISCUSSION

The present study demonstrates the high affinity of C.
burnetii for the gravid uterus and its abortifacient potential.
Experimental infection of pregnant mice with C. burnetii
resulted in abortion, stillbirth, and perinatal death. Antepar-
tum, necrosis of placental tissues, fibrinoid degeneration of
decidual vessels, and microthrombosis were observed, and
C. burnetii antigen was simultaneously demonstrated to be
present in abundance in the placenta. Surprisingly, fetal
tissues were devoid of C. burnetii antigen. However, off-
spring killed at day 9 of age exhibited lesion-associated
positive staining for C. burnetii antigen in the lung, liver, and
spleen.

Clinically, C. burnetii-infected pregnant mice displayed a
variety of failures in reproductive performance but also gave
birth normally. Similar observations have been reported for
rodents and other species infected with C. burnetii (14, 24,
28, 30, 40). Initial infection of the placenta occurred in the
decidua, and then the fetal compartment was invaded.
Localization of C. burnetii antigen in the labyrinthine pla-
centa indicates hematogenous dissemination of the organism
from the decidua to the fetal placenta. Once the organisms
had invaded the decidua and labyrinth, infection resulted in
uncontrolled growth and/or accumulation of C. burnetii in
the placenta. Comparative evaluation of the spleen and
placenta revealed that the former showed strong immunore-
activity as early as 3 days after infection. In contrast, in
the uteroplacental unit, a plateau of strong immunoreactivity
was present between 9 and 14 days after infection. More-
over, the intensity of the immunostaining in the uteroplacen-
tal region exceeded the intensity of staining in the spleen.
Despite strong immunoreactivity in the uteroplacental unit,
C. burnetii antigen was not demonstrated in fetal tissues,
indicating that the fetoplacental unit resisted vertical infec-
tion. Similarly, lack of fetal infection despite heavy placental
colonization was reported for mice inoculated with Chla-
mydia trachomatis (47).

The failure to find C. burnetii antigen in fetal tissue was
not due to the inadequacy of the method used, because C.
burnetii antigen was readily demonstrated in other organs of
the dam and in offspring at 9 days of age. However, lack of
fetal infection needs to be confirmed by more sensitive
techniques, such as the mouse infectivity test or polymerase
chain reaction. Neonatal infection with C. burnetii of the
offspring of latently infected guinea pigs and mice has been
described (44). The fact that C. burnetii antigen was found
in the lung, liver, and spleen of the offspring suggests that
aerosol infection occurred by inhalation of C. burnetii re-
leased from birth products. However, alimentary infection
after ingestion of contaminated milk (mammary glands were
not investigated) or fetal fluids followed by hematogenous
dissemination cannot be completely ruled out.

The role of the metrial gland and the GMG cells during
pregnancy is still undetermined, but it appears that they are
important in the immunology of the fetomaternal relation-
ship (12). Several studies suggested that GMG cells, descend-
dants of bone marrow cells morphologically resembling large
granular lymphocytes, may be natural killer-like cells (34).

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* Scoring: -, negative; +, weak, single focus; ++, moderate, multifocal; ++++, strong, multifocal.
* Values represent the mean score for all animals killed on the same day after infection.
* Values are for one animal only.
* Demonstrated in only one animal.
* N, tissue not present at time of examination.

TABLE 2. Spread and distribution of C. burnetii antigen in the uteroplacental unit and spleen of BALB/cJ (H-2b) mice after intraperitoneal inoculation on day 6 of gestation.
Decidual tissue is composed of leukocytes and stromal, macrophage-like, and GMG cells (20, 21). In the present study, *C. burnetii* antigen was found predominantly in macrophage-like decidual cells and labyrinthine trophoblasts. Interestingly, GMG cells were immunocytochemically negative for *C. burnetii* antigen, and only a few macrophage-like positive cells were demonstrated in the metrial gland. The significance of these observations and their relevance for the pathogenesis of *C. burnetii*-associated lesions in the gravid uterus remain to be determined.

The fetoplacental unit represents an immunologically privileged site, and several factors could account for the uncontrolled growth of *C. burnetii* at this location. Survival of the conceptus has been attributed to active systemic and local immune suppression as well as to the placental barrier (8). Luft and Remington (27) showed that, during pregnancy, peritoneal macrophages are defective in their ability to kill intracellular pathogens. However, in the present study, there was no evidence of systemic disturbance in macrophage function. *C. burnetii* organisms disseminated to and were cleared from the spleen as described for nonpregnant BALB/cJ mice (6a). Numerous suppressor substances and suppressor cells have been detected in the pregnant murine uterus, especially in the decidua (9, 23). Decidual cells may deliver inhibitory signals to macrophages, T cells, or both (8). Several immunosuppressive mediators, including a molecule related to transforming growth factor beta-2 that is released by non-T small lymphocytic suppressor cells and impairs the mobility of interleukin-2 receptors, have been described (10). Furthermore, decidual cells and macrophages suppress T-cell activity by release of prostaglandin E₂ (9). Accordingly, in vitro studies revealed that macrophage-like cells in uterine cell suspensions inhibit T-cell proliferation (19). In addition, nonspecific intrauterine suppression and differential regulation of cell proliferation might be a function of the metrial gland (12). Furthermore, active

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**FIG. 4.** Spread and distribution of *C. burnetii* in the spleen and uteroplacental unit. (A and B) at 3 days p.i., numerous immunopositive cells in the splenic red pulp (A) and few antigen-bearing cells in the decidua parietalis (arrows) (B). (C) At 9 days p.i., strong immunostaining in the decidua basalis and few foci of immunoreactivity in the labyrinthine placenta. (D) At 11 days p.i., strong immunoreactivity in the fetal and maternal compartments of the placenta. Abbreviations: D, decidua basalis; E, endometrium; G, giant-cell layer; L, labyrinth; M, myometrium; MG, metrial gland. ABC staining technique. Bars: (A) 150 µm; (B) 162 µm; (C and D) 200 µm.

**FIG. 5.** Appearance of *C. burnetii* antigen in the labyrinthine placenta 11 days after infection. (A) Numerous immunopositive mononuclear cells within dilated maternal vessel. (B) *C. burnetii* antigen in cytoplasmic vacuoles of trophoblasts (arrow) and within blood spaces (S). ABC staining technique. Bars, 12 µm.
local immunosuppression could be enhanced by the phase I cell-associated immunosuppressive complex of *C. burnetii* (48). Studies investigating infection of the murine uteroplacental region by *Listeria monocytogenes* found evidence that local immunoregulation, which normally prevents maternal antifetal response, also inhibits an effective antilisterial response (38).

The uterine lesions can be described as self-limiting, with complete resolution. Although the connection is highly speculative, the observed placental lesions are reminiscent of changes described as a local Schwartzman-like reaction following administration of endotoxin (13, 17, 29, 51). Some biological activities associated with gram-negative bacterial endotoxin are elicited by *C. burnetii* lipopolysaccharide (LPS) (5), but compared with other pathogens, *Coxiella* LPS can be classified as a poor endotoxin (2, 16, 33, 42, 43), and large quantities of purified *C. burnetii* LPS are necessary to induce endotoxic changes (31). Many biological activities once attributed directly to LPS are now known to be mediated by LPS-induced cytokines. Recent studies demonstrated production of tumor necrosis factor alpha by murine spleen and peritoneal exudate cells within 48 h after inoculation of a killed *C. burnetii* phase I whole-cell preparation (48). Since many macrophage-like decidual cells were immunocytochemically positive for *C. burnetii* antigen, it remains possible that cytokines secreted by these cells play a role in the development of *C. burnetii*-associated lesions in the placenta. Accordingly, in vitro studies with human decidual cells showed that these cells synthesize and secrete tumor necrosis factor alpha and prostaglandin F2α (7). Furthermore, application of LPS, tumor necrosis factor alpha, or interleukin-1α during pregnancy results in placental necrosis followed by fetal death and abortion (45). In addition, mice primed with *C. burnetii* extracts are highly susceptible to tumor necrosis factor-induced abortion (11).

However, further studies are necessary to investigate the pathogenesis of *C. burnetii*-associated lesions in the placenta. The susceptibility of different placental cell populations to this pathogen and infection-induced release of cytokines by these cells need to be evaluated.

**ACKNOWLEDGMENTS**

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**REFERENCES**


**FIG. 6. Distribution of *C. burnetii* antigen in the postgradiv uterus. At 28 days p.i., immunoreactivity is restricted to sites of previous placental attachment. Abbreviations: E, endometrium; M, myometrium; U, uterine lumen. ABC staining technique. Bar, 190 μm.**
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