Murine Granulated Metrial Gland Cells Are Susceptible to *Chlamydia psittaci* Infection In Vivo

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Received 16 February 1996/Returned for modification 10 April 1996/Accepted 28 May 1996

Granulated metrial gland (GMG) cells are the most numerous lymphoid cells in the uteroplacental unit in rodent pregnancy. In an experimental murine model of abortion-causing infection, we have studied the responses of GMG cells to *Chlamydia psittaci*. Chlamydial inclusions have been found within GMG cells, both in apparently healthy cells and in cells with degenerative changes. Establishing the existence of GMG cells infected by *C. psittaci* opens a new and interesting chapter in the study of these cells.

Granulated metrial gland (GMG) cells are large lymphoid cells which are a characteristic feature of rodent pregnancy (11). GMG cells differentiate in the decidua basalis and metrial gland in the uterine wall and appear as large rounded or ovoid

the uterine wall from a lymphoid precursor (6), but the lineage to which they belong is uncertain (12). It has been suggested that they are a type of NK cell (4), but more recently this has been questioned (12) and the possibility that they are a type of

FIG. 1. Paraffin wax section of metrial gland tissue taken from a mouse 5 days postinfection and reacted with a biotinylated anti-LPS antibody. GMG cells with numerous periodic acid-Schiff stain-positive granules and neutrophils are present. Positive reactions for LPS antigen are present in a GMG cell (arrow) and in neutrophils (arrowheads). The avidin-biotin-peroxidase complex and periodic acid-Schiff stain were used. Bar = 25 μ m.

cells (up to 50 μ m in diameter) containing numerous cytoplasmic granules that react positively with the periodic acid-Schiff stain (5, 13). Their numbers, distribution, and morphology vary according to gestational age (11). GMG cells differentiate in

natural cytotoxic cell has been put forward (2). GMG cells are able to kill some labyrinthine placental trophoblast cells (9, 10), and it has been suggested that they play a role in halting the transmission of viruses from the mother to the fetuses (16). Overall, however, although a variety of functions have been proposed for GMG cells, none has been substantiated and it is even suggested that the function of GMG cells varies according to the stage of gestation (3).

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FIG. 2. Transmission electron photomicrographs of *C. psittaci* inclusions in murine GMG cells 7 days postinfection; the sections were reacted with a biotinylated anti-LPS antibody. (a) GMG cell containing a young chlamydial inclusion. Bar = 3 μ m. (b) High magnification of panel a, showing a positive reaction for LPS in reticulate bodies and in the inclusion matrix. Bar = 1 μ m. (c) Vacuolated GMG containing a large chlamydial inclusion. Bar = 3 μ m. (d) High magnification of panel c, showing a positive reaction for LPS in EBs and in the inclusion matrix. Bar = 1 μ m.

FIG. 3. Electron micrograph of a neutrophil in a metrial gland from a 7-day postinfection mouse. The section was reacted with a biotinylated anti-LPS antibody and shows a positive reaction for LPS in areas of membrane-bound amorphous material (asterisks) and in an EB-like structure (arrowhead). Bar = 0.55 μ m.

The aim of this work was to study GMG cells in animals with an abortion-causing infection and to determine their response to the pathogen. We have, therefore, examined the susceptibility of mouse GMG cells to an obligately intracellular bacterium, *Chlamydia psittaci*, in an established model of chlamydiosis in pregnant mice (7); in this model, inoculation with 10^5 to 10^6 PFU of *C. psittaci* induces abortion by day 19 to 20 of pregnancy in nearly all pregnant mice. However, the pathogenesis of abortive chlamydiosis is not well known.

Infection during pregnancy by *C. psittaci* induces abortion in many mammalian species, including those of agricultural importance (15) and humans (1). These bacteria show a unique growth cycle including an infectious form, the elementary body (EB), which is initially taken into a susceptible cell, and an active metabolic form, the reticulate body, which divides by binary fission within the infected cell. The cycle is completed by the reorganization of the reticulate bodies into EBs and the release of the EBs, usually by lysis of the host cell. *C. psittaci* is able to infect a wide range of eucaryotic cells, but lymphocyte infection has not been reported. Since GMG cells are the most numerous type of lymphoid cell in the uterus and placenta during pregnancy, we were particularly interested in determining their susceptibility to infection, which would be pivotal if GMG cells have a role in limiting the passage of *C. psittaci* across the placenta to the fetus.

Adult (56- to 60-day-old) OF1 mice were obtained from the animal facility of the Institut National de la Recherche Agronomique, Nouzilly, France. Virgin females were mated overnight with males of the same strain; the presence of a vaginal plug the following morning indicated successful mat-

ing, and this day was designated day 0 of pregnancy. At day 11 of pregnancy, mice were inoculated intraperitoneally with 5×10^5 PFU of *C. psittaci* AB7, isolated from an ovine abortion (7), in 200 μ l of 0.1 M phosphate-buffered saline (PBS) (pH 7.2). On days 3, 5, and 7 postinoculation, three mice were killed by cervical dislocation. Of 10 infected control mice, which were not killed, 9 aborted at day 8 or 9 postinfection and 1 had a normal gestation period (21 days), but the pups died within 2 days of birth.

Implantation sites from the mice were fixed in 10% formaldehyde in PBS, dehydrated, and embedded in paraffin wax at 56°C for light microscopy. To visualize chlamydial antigen on paraffin sections (5 μ m thick), immunohistochemical staining was carried out with a biotinylated mouse monoclonal anti-chlamydial-lipopolysaccharide (LPS) primary antibody (8) at a 1/25 dilution and the avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, Calif.). A positive reaction was demonstrated by diaminobenzidine tetrahydrochloride precipitation. Sections were subsequently stained with hematoxylin and with the periodic acid-Schiff stain to visualize GMG cells.

For electron microscopy, implantation sites were rapidly excised and immersed in a mixture of 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M PBS. The metrial gland and decidua basalis were cut into small pieces and fixed in the same fixative for 24 h at 4°C. After fixation, tissues were dehydrated in ethanol and embedded in LR-White resin, soft grade (London Resin Company, Basingstoke, United Kingdom). Thick sections (1 μ m thick) were cut, stained with toluidine blue, and examined with a light microscope to locate GMG cells. Thin sections (80 nm thick) were then cut and immunostained. Immunogold staining of sections with the biotinylated anti-chlamydial-LPS antibody described above at a 1/25 dilution was carried out by a modification of a previously described method (8). Briefly, sections were collected on nickel grids and incubated for 1 h at 37°C with the primary biotinylated anti-LPS antibody. After several washes in PBS, the sections were incubated for 30 min at 37°C with streptavidin-gold (10-nm-diameter particles; Sigma, Madrid, Spain), washed in PBS, and counterstained in aqueous uranyl acetate and lead citrate.

Control sections were reacted either by the avidin-biotin-peroxidase complex technique or by the immunogold technique. These control sections either were treated with nonimmune mouse serum instead of the primary antibody or were sections of tissue from non-infected mice. These control sections were all negative.

In paraffin sections reacted with an anti-LPS antibody, chlamydial inclusions were observed within GMG cells located in the decidua basalis and metrial gland (Fig. 1). Chlamydial inclusions were found by day 5 postinoculation, but a higher proportion of GMG cells appeared to have chlamydial inclusions at day 7 postinoculation. Electron microscopy showed that some GMG cells, which generally appeared healthy, contained small or medium-sized chlamydial inclusions (Fig. 2a and b). However, other GMG cells contained large inclusions (Fig. 2c and d) with a lot of EBs, and these GMG cells showed degenerative changes, including cytoplasmic vacuolation and pyknosis of the nucleus. These findings suggest that the pathogen is able to infect mouse GMG cells and complete its growth cycle, which would lead to the lysis of the GMG cell and the release of EBs.

Few immunolabeled neutrophils were found in the decidua basalis and metrial gland at 3 days postinoculation, but they were numerous at 5 and 7 days postinoculation (Fig. 1). In paraffin sections, there was strong binding of the anti-LPS antibody to cytoplasmic components of the neutrophils. By electron microscopy, immunogold labeling of membrane-

bound amorphous material was found when sections were reacted with the anti-LPS antibody (Fig. 3), suggesting that the neutrophils were endocytosing chlamydiae to destroy them rather than being infected by the chlamydiae and providing a host environment in which the bacteria could complete their normal growth cycle. We did not find this kind of immunostained amorphous material in GMG cells.

In the current study, mice were infected at day 11 of pregnancy and chlamydia-infected GMG cells were found at 5 days postinoculation or later. It has been suggested that GMG cells function to remove aberrant trophoblasts, particularly during the period of placental development (9, 10). Since the *C. psittaci*-infected GMG cells found in this study were first seen on day 16 of pregnancy, a time when the GMG cell population is in decline (14), it is possible that GMG cells were in a postfunctional state. It will be important, therefore, to determine if infection of GMG cells occurs at earlier stages of pregnancy, when GMG cells are more active (11). Nevertheless, the infection of mouse GMG cells with *C. psittaci* and the maintenance of the growth cycle of *C. psittaci* in these cells, as reported here, open a new line of study, particularly because GMG cells are the principal maternal lymphoid cells in the uteroplacental unit in pregnancy and because infection of lymphoid cells by *C. psittaci* has not been reported to date.

Part of this work was supported by a grant from The Wellcome Trust (Ref. 044087). A. J. Buendía was the recipient of a predoctoral grant from the Universidad de Murcia, Spain.

We thank M. C. Martínez for her excellent technical assistance, C. De Jodar for photography, and A. Souriau for his help with the biotinylation method for the anti-chlamydial-LPS monoclonal antibody.

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