Experimental Infection of the Genital Tract of Female Grivet Monkeys by Mycoplasma hominis

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Mycoplasma hominis, a common inhabitant of the mucosae of the genitourinary tract of human and nonhuman primates, was inoculated directly into the uterine tubes of five laparotomized grivet monkeys. A self-limiting acute salpingitis and parametritis developed within a few days in all animals. Although there were no clinical signs of overt disease, the gross pathology was characterized by pronounced oedematous swelling and hyperaemia of the tubes and parametria. Microscopically, cellular infiltrations of lymphocytes and some polymorphonuclear leukocytes were found in the acute phase in the subserosa and muscularis of the tubes and in the parametria. Granulation tissue and fat necrosis appeared at a later stage in the parametria. The infection was associated with a marked antibody response and a moderate rise of the erythrocyte sedimentation rate and leukocyte counts. The capability of M. hominis to produce salpingitis and parametritis in a nonhuman primate would seem to add rather significantly to the available evidence suggesting an etiological role of this organism in inflammatory diseases of the internal female genitals of humans.

The first isolation of mycoplasmas from humans was made in 1937 from a Bartholin gland abscess (7). Since then, two mycoplasmal species, Mycoplasma hominis and Ureaplasma urealyticum, have been shown to be very common inhabitants of the human genitourinary mucous membranes, and many attempts have been made to determine the pathogenic potential of these organisms in relation to inflammatory diseases of the genital tract of males and females (10–12, 27, 28). In females, the possible etiological implication of these organisms in salpingitis and related disorders has attracted particular interest. For obvious reasons, isolations made from the cervix and vagina do not provide conclusive evidence as to a causal relationship of the organisms to infection of the uterine tubes. Although recovery of mycoplasmas directly from the tubes or from tubo-ovarian and pelvic abscesses has occasionally been reported (11, 12), it has generally been difficult to obtain specimens from the uterine tubes in the acute stage of salpingitis for cultivation attempts. This object could be achieved, however, with the introduction of laparoscopy as a direct method of diagnosing salpingitis (19). The circumstantial evidence obtained by this means in favor of the concept that M. hominis, and possibly U. urealyticum, may be the cause of some cases of salpingitis has gained additional support from a number of serological studies (15, 20, 22). Nevertheless, the true significance of mycoplasmas as causal agents of salpingitis is still the subject of discussion.

The purpose of this study was to determine whether salpingitis and inflammatory lesions of other tissues of the internal female genitals could be produced experimentally by M. hominis in a nonhuman primate.

MATERIALS AND METHODS
Organism. M. hominis D1887, isolated in this laboratory from the cervix of a patient with acute salpingitis, was used in all the experiments. It had been cloned only once after the primary isolation and was stored at −70°C. For preparation of the inoculum, the organism was subcultured twice in a liquid medium (B-medium) consisting of heart infusion broth (Difco) enriched with 20% (vol/vol) horse serum and 10% of a 25% (wt/vol) solution of yeast extract. The second subculture was made in 300 ml of medium and harvested in the late log growth phase by centrifugation (10,000 rpm/20 min) and suspension of the pellet in 5 ml of phosphate-buffered saline, pH 7.2. The concentrated suspension contained about 10⁵ colony-forming units per ml. Portions (1 ml) were frozen and stored at −70°C. The number of colony-forming units of the individual lots was redetermined before each experiment to ensure that no significant loss of viable units had occurred during storage. In one experiment, the culture used for inoculation was derived from an isolate of M. hominis that had been recovered from the uterine tube of another monkey after experimental infection with strain D1887.
Animals. Six female grivet monkeys (Cercopithecus aethiops), weight 1.5 to 2.2 kg, were used. The animals had been captured in East Africa and kept in quarantine for at least 6 weeks at Statens Seruminstitute in Copenhagen, where they had been tested for enteric pathogens (Salmonella and Shigella spp.) and for tuberculosis by the tuberculin test. Throughout the experimentation period the monkeys were caged individually in an isolation room. They were fed a commercial primate food and were supplied with fresh fruit and water ad libitum.

Preinoculation testing. Throat, vaginal, and rectal swabs were obtained three times, at intervals of 2 to 3 days, while the animals were anesthetized with ketamine chloride (Ketalar, 5 mg/kg). Cultivations were made to ascertain that the monkeys did not harbor M. hominis or U. urealyticum in any of these sites. In addition, vaginal swabs were cultivated for aerobic and anaerobic bacteria and for Trichomonas vaginalis (Diamond medium [6]). A retrospexlusion of Chlamydia trachomatis infection was attempted at a later stage by cultivation of cervical swabs in cycloheximide-treated McCoy cells (25). Blood samples for serology, leukocyte countings, and determination of the erythrocyte sedimentation rate (ESR) were taken by inguinal puncture of the femoral artery while the monkeys were under anesthesia. The rectal temperature was recorded twice daily by a thermistor.

Experimental infection. Infection was induced by inoculation directly into the uterine tubes by laparotomy performed while the monkeys were under anesthesia with phenylcycline hydrochloride (Sernylan, 20 mg/ml), 0.15 ml; chlorpromazin (0.25% solution), 0.5 ml; and atropin (0.1% solution), 0.2 ml.

Surgery was performed under aseptic conditions. The abdomen was shaved, washed with soap, and disinfected with a 2% iodine tincture. The skin was covered with a sterile surgical drape (Steri-drape). The laparotomy was made by a paramedian incision, after which 0.2 ml of the mycoplasma suspension was injected through the wall of the lateral part of each uterine tube by using a 0.8-mm needle. It was endeavored to deposit the inoculum into the lumen of the tubes. In one monkey included as a control for the whole series of experiments, phosphate-buffered saline was injected instead of the mycoplasma suspension. The abdominal wall was closed by a suture in three layers, and the wound was covered with spray-plaster (Nobucutan). To minimize the risk of bacterial infection, daily injections of methicillin (Lucopenin, 60 mg/kg) were initiated on the day of operation and were continued until day 5 postoperation. The surgery was tolerated very well by the monkeys.

Assessment of lesions and collection of specimens. Laparotomy as described above was performed, with minor temporal variations, on days 3, 7, 12, 20, and 35 postinoculation (p.i.) to follow the development and regression of inflammatory lesions and to collect specimens. Swabs for recovery of mycoplasmas were taken from the vagina, fimbriae, uterine tubes, uterine cavity, and parametria, and swabs for bacterial cultivation were taken from the fimbriae, uterine tubes, uterus, and the peritoneal serosa covering the intestines. Biopsies were taken from the tubes and parametria by means of 2-mm surgical ear-forceps and from the uterus by a 3-mm skin drill.

Culturing of specimens. For cultivation of M. hominis the swabs were inserted immediately into 1.7 ml of the liquid B-medium containing penicillin G, 400 IU/ml, and thallium acetate, 0.01% (wt/vol). After incubation at 37°C for 3 days, subcultivation was made into (i) liquid B-medium, (ii) liquid B-medium containing 0.3% of arginine, and (iii) a duplicate set of solid B-medium plates prepared by replacing heart infusion broth with heart infusion agar (Difco). Again, subcultivation onto solid medium was made from the secondary liquid cultures after 3 days of incubation. One set of agar plates was incubated at 37°C in candle jars, and the other set was incubated in an atmosphere of 95% N₂ and 5% CO₂. The plates were read under a stereomicroscope after 3 days and, if negative, were reexamined at intervals during incubation for another 7 days. Identification of mycoplasma isolates was made by the growth inhibition (2) and indirect epi-immuno- fluorescence tests (26).

Cultivations for bacteria were made in brain heart infusion broth (Difco) and on blood agar, lactose bron- thomy, and chocolate agar plates.

Histology. The tissue biopsies were fixed in 10% Formalin and processed by routine histological methods, including staining with hematoxylin and eosin.

Serology. The indirect hemagglutination (IHA) test was performed by the method of Krosgaard- Jensen, by using Formalinized sheep erythrocytes sensitized with the supernatant fluid after centrifugation of sonically treated antigen (14).

RESULTS

All five monkeys that were experimentally infected by inoculation of M. hominis into their uterine tubes presented gross pathology as well as histological evidence of acute inflammatory lesions of the upper genital tract. The entire course of infection was essentially the same in all experimental animals, including the one which received the monkey-passaged M. hominis inoculum. Clinically, none of the monkeys presented any remarkable signs of disease, their general health condition remaining fairly unimpaired.

Gross lesions. Signs of an acute inflammatory reaction were apparent as early as day 3 p.i. Characteristically, the lateral part of the uterine tubes were swollen and reddened, whereas at this stage the medial part and the parametria looked normal. On day 7 p.i., the tubes showed a pronounced swelling and hyperemia in their entire length, and the parametria were moderately to markedly oedematous. After 12 days the signs of inflammation of the tubes were decreasing, but the parametria still showed pronounced swelling. During the following 2 to 3 weeks there was a further marked regression of the inflammatory lesions, and the upper genital tract was virtually normal in appearance 4 to 5 weeks after
inoculation of the mycoplasmas.

No exudation was observed from the tubes at any stage of infection. The uterus showed only slight signs of inflammation, and the ovaries were invariably normal without oedema or reddening. Cystic structures did not develop either in the tubes, ovaries, or parametria.

**Histology.** Pronounced inflammatory changes were found microscopically in the uterine tubes and parametria of all five monkeys inoculated with *M. hominis* (Fig. 1 and 2).

The lumen of the tubes always had a normal appearance without any exudate or inflammatory cells. The mucosa was likewise practically intact except for an occasional slight hyperemia and lymphoid infiltration. The most notable finding in the tubes was an intense infiltration in the subserosa of lymphoid cells together with an increased number of polymorphonuclear leukocytes. In most cases, the cellular infiltration extended into the peripheral layer of the muscularis.

The inflammatory lesions of the parametria were characterized by marked oedema and hyperemia, accompanied by cellular infiltrations consisting mainly of great numbers of lymphocytes, but also of some heterophilic granulocytes. In most cases, fat necrosis and granulation tissue appeared in the parametria about days 10 to 12 p.i.

**Body temperature.** No significant rise of the rectal temperature was observed in relation to mycoplasma infection.

**Hematology.** The ESR showed a moderate rise on days 3 to 12 p.i. (Fig. 3). The number of leukocytes exhibited a slight decrease on day 3, followed by a moderate increase on days 7 to 14 p.i. (Fig. 3). Differential countings showed no consistent changes.

**Isolation of *M. hominis.*** This could be recovered regularly from the fimbriae, tubes, parametria, and the uterine cavity on days 3 and 7 p.i. Swabs taken from the said tissues during subsequent laparotomies usually yielded no growth of mycoplasmas, although in one case *M. hominis* was isolated from the uterus on day 10, and in another case from the fimbriae on day 17 p.i. From the vagina, *M. hominis* could be isolated from day 3 until about 3 weeks (four cases) and 3.5 month (one case) p.i.

With one exception, no growth of bacteria was obtained from swabs taken in connection with laparotomy. *Chlamydia* could not be isolated from cervical swabs.

**Serology.** Although none of the monkeys had detectable IHA antibodies in preinoculation sera, such antibodies developed 7 to 12 days p.i. A fourfold rise was observed in all monkeys during the course of the infection, with maximum titers of 160 to 640. This titer level persisted for more than 3 months in every case (Fig. 3).

**Control monkey.** The body temperature, ESR, and leukocyte counts of the monkey inoculated with phosphate-buffered saline remained unchanged within preinoculation values. Macroscopic signs of inflammation of the internal genitals were not demonstrable at any time. Microscopically, very minimal inflammatory changes were seen confined to the tubes. *M. hominis* could not be isolated, and no antibodies developed against this organism, although the animal was caged in the same room as the experimentally infected animals (Fig. 3).

**DISCUSSION**

Although *M. pneumoniae*, the major etiological agent of primary atypical pneumonia, is the only mycoplasma of proven pathogenicity to humans, the possible role of other mycoplasmal species in human disease has been the subject of numerous investigations during recent decades (12, 28). In the Introduction, mention was made briefly of findings that strongly suggest that *M. hominis*, and possibly *U. urealyticum*, may be etiologically involved in salpingitis and other inflammatory conditions of the female genital tract. The studies by Mårdh and Weström (22; P.-A. Mårdh, M.D. thesis, University of Lund, Lund, Sweden, 1972), who obtained samples for cultivation attempts directly from inflamed salpinges through the laparoscope, are particularly noteworthy. In 4 out of 52 cases of acute salpingitis such specimens yielded growth in pure culture of *M. hominis*, whereas in 2 cases *U. urealyticum* was recovered. Antibodies against *M. hominis* were demonstrated by IHA in 54% of the 52 patients, with a significant change of the titer in 7 out of 29 patients from whom more than one serum specimen was collected during the course of the disease (20). The demonstration, in 34% of the total number of patients, of markedly increased serum levels of immunoglobulin M (17) was in accordance with similar findings in patients suffering from *M. pneumoniae* pneumonia (1, 9). The quite high incidence of *M. hominis* antibodies recovered by Mårdh and Weström in their salpingitis material, as opposed to a low incidence in healthy controls, provided further support for the significance of similar observations previously made by other authors (15, 22).

Previous attempts at proving the pathogenicity of *M. hominis* and other mycoplasmas of human provenance have included, on a few occasions, experimental infection of human volunteers (3, 23, 29). For obvious reasons, it would be out of the question to follow this approach as a possible means of reproducing salpingitis under experimental conditions. The choice in the
Fig. 1. Histopathological appearance of monkey uterine tube inoculated with $0.2 \times 10^6$ colony-forming units of M. hominis, D1887. (A) Normal tube at day of inoculation. (B) Day 3 p.i., oedema and slight inflammation in mucosal folds, pronounced inflammation in muscular coat and serosal covering. (C) Day 12 p.i., inflammatory changes regressing. (D) Day 21 p.i., inflammatory lesions subsided except for moderate changes in the serosal coat. Stained with hematoxylin and eosin, x52.
present study of a nonhuman primate as an alternative experimental model was motivated, not only by the wish to use an experimental animal that is phylogenetically relatively closely related to humans, but also by the fact that the normal mycoplasmal flora appears to be shared, to a wide extent, by human and nonhuman primates. Thus, *M. hominis* has been isolated.
FIG. 2. Histopathological appearance of monkey parametrium after inoculation of the uterine tube with M. hominis D1887. (A) Day 3 p.i., slight superficial inflammation. (B) Day 7 p.i., pronounced inflammatory changes. (C) Day 12 p.i., pronounced signs of inflammation, including marked necrosis of fatty tissue. (D) Day 21 p.i., cellular infiltration regressed. Granulation tissue and commencing fibrosis can be noted. Stained with hematoxlin and eosin, x52.
from the oropharynx and the lower genital tract of a variety of primate species in captivity (4, 5, 16, 21).

The production by inoculation of *M. hominis* in grivet monkeys of a self-limiting acute salpingitis and parametritis associated with a marked...
antibody response, together with a moderate rise of the ESR and leukocyte counts, would seem, in our opinion, to add rather significantly to the assumption that *M. hominis* is also capable of producing salpingitis in humans under natural conditions.

On the basis of gross pathology and histology, non-tuberculous salpingitis in humans may be divided into two main groups (24). One type, which is caused by *Neisseria gonorrhoeae*, is characterized by a moderate swelling and reddening of the uterine tubes, and the parametria usually look practically normal. The lumen of the tubes is distended with a purulent exudate, which may also escape the abdominal ostia and produce pelvic peritonitis and abscesses. The mucous membrane of the tubes is swollen and hyperaemic. Microscopically the epithelium shows cloudy swelling of the cells and patchy destruction. The subepithelial tissue is infiltrated with leukocytes, chiefly of the polymorphonuclear variety. The pathogenesis of the gonorrheal salpingitis is generally regarded to depend on ascending infection from a primary lesion of the lower genital tract, the gonococci reaching the endosalpinx via the uterine mucosa.

In non-gonorrheal salpingitis, the inflammatory swelling, which also involves the parametria, is usually much more pronounced than in the pyogenic infection. The swelling of the tubes is due to an enormous oedematous thickening of the tube wall, whereas there is no exudate in the lumen. The histology is characterized by a normal epithelium and marked oedema and other acute inflammatory changes of the subserosa and muscularis of the tubes. The parametria, likewise, show intense oedema, hyperemia, and infiltration with leukocytes. This type of salpingitis and parametritis may occur as a postpartum or postabortive complication, or result from

**Fig. 3. Antibody titers by IHA, ESR, and leukocyte counts (LC) in: (○) control monkey, and (◆ □ △ ▲) monkeys inoculated with *M. hominis* D1887 into the uterine tube (mean ± standard error of the mean). ■*, Not done.**
surgical procedures such as cauterization of the cervix or uterine curettage. In both sets of conditions, microorganisms are believed to gain entrance to the tissues through lesions of the cervical or endometrial epithelium and to spread to the tubes, parametria, and broad ligaments via blood vessels and lymphatics.

It will be seen from that described above that the pathology of the experimentally induced *M. hominis* genital tract infection of monkeys closely resembles the latter type of salpingitis and parametritis described in humans. Other possible causes of this type of inflammation include staphylococci and streptococci. The particular importance of *C. trachomatis* as another etiological agent of acute salpingitis is borne out by recent investigations (8, 13, 18). Altogether, gonococci would seem to be a relatively less frequent cause of salpingitis and related disorders in the genital tract. The production of *M. hominis* infection of the genital tract by direct inoculation into the uterine tubes is admittedly highly artificial. However, further experiments are in progress in this laboratory with the purpose of reproducing the disease under conditions simulating more closely, the natural infection.

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**LITERATURE CITED**

