EXPERIMENTAL INFECTION OF THE UPPER GENITAL TRACT OF FEMALE GRIVET MONKEYS WITH *MYCOPLASMA FERMENTANS*

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PLATES VI–VII

MYCOPLASMA FERMENTANS represents 2% or less of the mycoplasma flora of the lower urogenital tract of man (Black and Rasmussen, 1968; Mårdh, Nilsson and Bjelle, 1973). It has been isolated on one occasion from an inflamed uterine tube (Freundt, 1953), but the possible significance of this species as an aetiological agent in gynaecological inflammatory disease requires further study. The very frequent association of *M. fermentans* with rheumatoid arthritis reported by Williams, Brostoff and Roitt (1970) remains largely unconfirmed, although the organism has been isolated from the synovial fluid of patients with rheumatoid and non-rheumatoid arthritis (Mårdh *et al.*, 1973). The toxic effect of *M. fermentans* membrane fractions, when inoculated intraperitoneally in high concentrations into mice (Gabridge and Murphy, 1971; Gabridge, Yip and Hedges, 1975), provides another impetus for studying the potential pathogenicity of this organism.

In an earlier study, female grivet monkeys proved suitable as an experimental model for infection with *Mycoplasma hominis* (Møller *et al.*, 1978; Møller and Freundt, 1979). The present paper describes the experimental production of salpingitis and parametritis by *M. fermentans* in grivet monkeys, by the same techniques.

MATERIALS AND METHODS

M. fermentans. Two strains were used. Strain S38 was isolated in 1953 from the uterine tube of a patient suffering from recurring salpingitis (Freundt, 1953). After primary isolation and a few subsequent culture passages, the strain was stored in the lyophilised state at 4°C. Upon revival of the lyophil in 1977, cloning was performed three times and the identity of the strain was confirmed as *M. fermentans* by growth-inhibition and epi-immunofluorescence tests. Strain D1882 was isolated in this laboratory from the cervix of a 15-year-old girl attending a clinic for sexual guidance; it was cloned once. For preparation of inoculum, the organisms were subcultured twice in a liquid B-medium (Freundt, Ernø and Lemcke, 1979); the second subculture, in 300 ml of medium, was harvested, in the late log phase of growth, by centrifugation (10 000 r.p.m. for 20 min.) and the pellet of organisms was resuspended in 5 ml of PBS, pH 7.2. The concentrated suspension contained approximately 10⁸ colony-forming units (c.f.u.) per ml. Portions of 1 ml were frozen and stored at –70°C. The number of c.f.u. per ml of the individual lots was redetermined before each experiment.

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Animals. Nine female grivet monkeys, weight 1.5–2.2 kg, were used. They had been imported from East Africa, but before experimental infection they were kept in quarantine for at least 6 weeks and tested for *Salmonella* and *Shigella* and by the tuberculin test. During the experiments the animals were caged individually in an isolation room, as described earlier (Møller *et al.*, 1978).

Preinoculation testing. Cultivation experiments were made to exclude spontaneous colonisation of the monkeys, particularly with *M. hominis*, *M. fermentans*, *M. primatum*, and *Ureaplasma urealyticum*; specimens were taken from the pharynx, urethra, vagina, and rectum. This and other pre-inoculation testings were performed essentially as described by Møller *et al.* (1978).

Experimental infection. This was attempted by three different routes of inoculation: (A) monkeys I, II, and III were given injections of 0.4 ml of mycoplasma suspension directly into both uterine tubes exposed by laparotomy (Møller *et al.*, 1978), I and II with *M. fermentans* strain S38, and III with strain D1882; (B) monkeys IV and V were given 0.4 ml of the S38 suspension into the uterine cavity, by injection through the cervical canal with a 0.4-mm syringe; the cervix was not dilated; (C) in the case of monkeys VI and VII, the isthmus of the uterine tubes was closed by ligatures at laparotomy and strain S38 was injected into the uterine cavity; this was followed by dilatation of the cervix and curettage of the endometrium, as described in detail previously (Møller and Freundt, 1979).

Controls. Monkey VIII was given an injection of PBS directly into the lumen of the uterine tubes at laparotomy, and monkey IX was treated like monkeys VI and VII except that PBS was injected instead of mycoplasmas.

For all surgical procedures, the monkeys were anaesthetised with phenylclidine hydrochloride (Sernylan, 20 mg/ml), 0.15 ml; chlorpromazin (0.25% solution), 0.5 ml; and atropin (0.1% solution), 0.2 ml.

Assessment of lesions and collection of specimens. All monkeys were laparotomised 5, 10, 23, and 40 days postinoculation (p.i.) in order to examine the abdominal sexual organs for gross lesions, and to collect biopsy and swab specimens from the uterus, uterine tubes, fimbriae, parametria, and the intestinal serosa. Swabs were taken from the vagina at intervals from the 5th day until about 3 months p.i.

Culturing of specimens. Cultivation for *M. fermentans* and bacteria was performed as described for *M. hominis*. (Møller *et al.*, 1978).

Histology. Tissue biopsies were fixed in 10% formalin, embedded in paraffin, and stained with haematoxylin and eosin for histological examination.

Serology. Determination of antibodies was carried out by the indirect haemagglutination (IHA) test, with fresh sheep erythrocytes sensitised with the supernatant fluid obtained by centrifugation of sonicated antigen (Krogsgaard-Jensen, 1971).

RESULTS

None of the seven female grivet monkeys experimentally infected with *M. fermentans* showed any clinical signs of inflammation. Body temperature and leukocyte counts remained normal. The ESR showed a moderate rise when tested on days 5 and 10 p.i.

Gross lesions

On laparotomy, monkeys I, II, and III, which had been given injections of mycoplasmas directly into the uterine tubes, and monkeys VI and VII, which had received their injections into the uterine cavity itself, followed by curettage, showed obvious signs of inflammation only of the genital organs, at 10 and 23 days p.i. The parametria were moderately swollen and the tubes were slightly

oedematous and showed moderate hyperaemia. No exudate or cystic structures developed. The uterus and the ovaries were invariably normal.

Monkeys IV and V, which had been given mycoplasma injections into the uterine cavity without curettage, showed no signs of inflammation of the upper genital tract or elsewhere, throughout the observation period.

The control monkeys (VIII and IX) maintained an apparently normal genital tract throughout the experimental period.

The joints of the extremities of the monkeys inoculated with *M. fermentans* were regularly examined for swelling and redness, but with negative results.

Histology

Monkeys I, II and III and monkeys VI and VII demonstrated pronounced inflammatory lesions in the parametria and in the subserosa of the fallopian tubes. The most notable findings in the parametria were oedema and hyperaemia appearing on day 5 p.i., with infiltration by moderate numbers of lymphocytes and polymorphonuclear granulocytes into the tissues; in some areas there was marked fibroblastic proliferation and necrosis of fatty tissue at 10 and 23 days p.i. (fig. 1). The inflammatory lesions in the fallopian tubes were characterised by moderate infiltration of lymphoid cells into the subserosa, with slight oedema; there was no cellular infiltration of the muscularis layer or of the mucosa, the epithelium was intact and there was no exudate in the lumen. The inflammatory reactions were most pronounced on day 10 p.i. (fig. 2), but by day 23 only slight cellular infiltration remained in the parametria and the uterine tubes were practically normal; by day 40 all signs of inflammation had disappeared.

Monkeys IV and V and monkeys VIII and IX had no or only minimal inflammatory changes, confined to the subserosa of the tubes and parametria.

Isolation of mycoplasmas and bacteria

M. fermentans was recovered from the uterus and the vagina of monkey II, and from the fimbriae of monkey V, on day 5 p.i. but not later. From the other four monkeys infected with *M. fermentans* strain S38, the organism could be recovered only from the vagina on day 5 p.i. Monkey III, inoculated with *M. fermentans* strain D1882, yielded no growth from the internal genital organs; however, vaginal cultures from this monkey were heavily overgrown by bacteria.

Significant bacterial growth was not obtained from the swabs taken from any of the monkeys at laparotomy.

Serology

None of the monkeys had detectable IHA antibodies to *M. fermentans* in their preinoculation sera. Significant levels of antibody developed in monkeys I, II and III and in monkeys VI and VII during the course of the infection,

TABLE

Development of antibody in grivet monkeys infected with M. fermentans by various routes of inoculation

Route of inoculation	Monkey no.*	Indirect-haemagglutination (IHA) antibody titre at intervals (days) after infection					
		0	5	10	23	46	60
Directly into the uterine tube	I	≤20	≤20	160	40	≤20	≤20
	II	≤20	≤20	160	80	80	ND
	III	≤20	≤20	40	80	≤20	ND
Into the uterine cavity without curettage	IV	≤20	≤20	≤20	≤20	≤20	≤20
	V	≤20	≤20	≤20	≤20	≤20	≤20
Into the uterine cavity with curettage	VI	≤20	≤20	80	80	≤20	ND
	VII	≤20	≤20	80	80	≤20	≤20
Controls	VIII	≤20	≤20	≤20	≤20	≤20	≤20
	IX	≤20	≤20	≤20	≤20	≤20	≤20

ND = Not done.

* Monkeys I, II and IV–VII were infected with strain S38, and monkey III with strain D1882; the control monkeys were given only PBS.

reaching maximum titres of 80–160 (table). There was no detectable antibody to *M. fermentans* in monkeys IV and V, or in the control monkeys, during the observation period (table).

IHA antibodies to *M. hominis* were not detected in any of the monkeys at the start of the experiment and did not appear subsequently.

DISCUSSION

M. fermentans is a rather uncommon inhabitant of the urogenital tract of man. Thus, Braun *et al.* (1970) did not isolate it from urine or cervical specimens from any of 568 pregnant women, and Black and Rasmussen (1968) found that only 2% of *Mycoplasma* strains isolated from the urethra of 275 men were *M. fermentans*. In a single report, this micro-organism has been isolated in pure culture from the uterine tube of a patient suffering from subacute salpingitis (Freundt, 1953). In the present study, five out of seven monkeys given injections of *M. fermentans* into the genital tract developed, within a few days, acute inflammation of the upper genital tract accompanied by an antibody response. Three of the five had been given their injections directly into the uterine tubes, and the other two into the uterine cavity followed by curettage. On the other hand, two monkeys given their mycoplasma injections into the uterine cavity through the cervical canal, without subsequent curettage, failed to develop any signs of inflammation. The fact that an inflammatory reaction occurred only when injection of the intrauterine cavity was followed by curettage indicates that spread of *M. fermentans* to the parametria and uterine tubes must have taken place through blood vessels and lymphatics. The lesions produced by *M. fermentans* were essentially the same as those observed in experimental *M. hominis* infection, with similar routes of

inoculation, but the antibody response was generally of shorter duration (Møller *et al.*, 1978; Møller and Freundt, 1979).

The possible association of *M. fermentans* with arthritis received no support from the present study.

Although, to our knowledge, there is only one previous report of a possible association between *M. fermentans* and upper genital-tract infection (Freundt, 1953) our results may provide a stimulus to further clinical investigations in man. Such studies are in progress in our laboratory.

SUMMARY

Mycoplasma fermentans inoculated directly into the uterine tubes of female grivet monkeys produced a self-limiting acute salpingitis and parametritis. The inflammation was accompanied by a significant rise in the titre of specific indirect haemagglutinating antibodies. Inoculation of *M. fermentans* into the uterine cavity through the cervical canal without dilatation of the cervix produced practically no signs of inflammation and no antibody response. However, when the intrauterine inoculation of mycoplasmas was followed by curettage of the endometrium, in animals whose uterine tubes had been closed by ligatures, pronounced upper genital-tract inflammation developed, together with a significant antibody response.

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FIG. 1.—Histopathological appearance of the parametrium of monkey no. VI, at intervals after inoculation of *M. fermentans* into the uterine cavity followed by curettage. (A) Day of inoculation, showing normal parametrium. (B) Day 5 p.i., showing marked inflammatory changes. (C) Day 10 p.i., showing marked inflammation, with fibrosis of the tissue. Haematoxylin and eosin (HE). $\times 90$.

Mycoplasma INFECTION OF MONKEY GENITAL TRACT

FIG. 2.—Histopathological appearance of the uterine tube of monkey no. VI, 10 days after inoculation of *M. fermentans* into the uterine cavity, showing moderate inflammation of the subserosa; the muscularis layers and the epithelium are normal. HE. $\times 90$.