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THE VAGINAL FLORA IN IDIOPATHIC REPRODUCTIVE TRACT DISEASES OF WOMEN AND AN ANIMAL MODEL

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SUMMARY

This paper describes the results of a survey of over 380 women of child-bearing age who were assessed for the cause of their respective pelvic inflammatory complaints or women who presented without health complaints. The data show a strong correlation between infection with *Ureaplasma urealyticum* and chronic vaginal discharge and inflammation in the presence of an apparently normal population of vaginal lactobacilli. A suggestion of association with complaints consistent with endometriosis with *U. urealyticum* infection is noted and a possible murine model for this disease is presented.

INTRODUCTION

I wish to present some work done over a period of years, first with Miles Laboratories and then at the Trudeau Institute in Saranac Lake, New York, and also mention something dear to the hearts of Prof. David Taylor-Robinson and me: An animal model for urogenital mycoplasmosis.

The original objective of the program and team I led was to develop an antibody-based diagnostic kit that could be used in a clinician's office to diagnose gonorrhoea and other common causes of pelvic inflammatory disease (PID). A subsequent objective was to ascertain whether ureaplasmas had a causal relationship to endometriosis, a subject I would like to hear discussed during the symposium although there are no hard data in the literature yet to support such a relationship.

The approach we used was to collect vaginal fluids from patients with suspected gonorrhoea, undiagnosed vaginal infections, or women free of vaginal infection, according to the clinical judgement of the physician. The objective was to develop an antibody-based diagnostic test which is why the focus was originally on the vaginal washout fluids.

METHODS

The clinicians were provided with a kit of four 6" sterile cotton swabs, two glass slides and a slide mailer, a disposable 10 cc syringe and a tube containing azide to prevent destruction of protein (antibody), sterile pyrogen-free saline, and bacteriological media (Transgrow for gonococci, Kupferberg's for trichomonads, Nickerson's for *Candida*, and Shepard's for ureaplasmas/mycoplasmas). Assessments were also made, based on patient histories, which

Table 1: Sample population (all patients were aged 15-50 years)

161 patient samples from 3 Ob/Gyn clinics in northern Indiana
170 patient samples from 1 Ob/Gyn clinic in northern New York
58 patient samples from a County Health Department clinic in Indiana

are not the subject of this paper and will not be presented. Maurice Shepard advised on the cultivation of the mycoplasmas (some in the audience may remember that Dr. Shepard's original work on ureaplasmas was done on U.S. Marines at Camp Lejeune in North Carolina).

The procedure was to ask the clinicians to take the specimens, including cervical mucus specimens, put each of two swabs directly into Shepard's or Kupferberg's media, use a third swab to directly inoculate the Transgrow and Nickerson's media and maintain the media at room temperature until processing later in the day. One swab was used to streak each of two slides that were to be Gram-stained or used in fluorescent antibody assays. For the vaginal wash fluid, the physicians were asked to gently irrigate the vagina with 10 cc of saline and collect the fluid into the tube containing azide; this fluid was then refrigerated until processing. The immunological aspects of the study were not as successful as had been hoped and these data are not included in this presentation. The sample population included 161 women seen at three OB/GYN clinics in northern Indiana and 58 patients seen at a county health department clinic, where we saw most of the gonorrhoea. In subsequent studies in association with an OB/GYN clinic in northern New York, we had 170 patients sampled. The patient population ranged in age from 15 to 50; most of the women were in their twenties and thirties.

RESULTS

The sample population is summarised in Table 1 and the number of gonorrhoea cases are shown in Table 2; these are not extraordinary. Sixty cases of candidosis were observed and sixteen cases of trichomoniasis, two of which had a mixed *Candida* and *Trichomonas vaginalis* infection (Table 3). Sixty-six patients had no complaints; these were individuals who came into the clinic for PAP smears, annual physicals, or were in the early stages of pregnancy. Thirty-seven of the patients in the population presented with vaginitis/cervicitis as clinically diagnosed.

With respect to the subject of our symposium, it was interesting that *Candida*, unlike *Trichomonas*, gonorrhoea, and other PID agents which were present with a mixed vaginal flora, 60% of the *Candida* cases were observed in women in whom there was a rich popu-

Table 2: Gonorrhoea cases

11 (58) GC cases from Indiana Health Dept.
5 (161) GC cases from Indiana clinics
8 (170) GC cases from NY clinic

Table 3: Pelvic inflammatory disease (Indiana clinics)

- 60 cases of candidosis and 16 cases of trichomoniasis (2 patients with double infections)
- GC virtually limited to county health clinic
- 66 patients presented with no complaint
- 137 patients presented vaginitis/cervicitis

lation of lactobacilli. What was most interesting to me was the observation that, in the presence of a normal population of lactobacilli, and no other known PID agents, women with a chronic vaginal discharge were positive for ureaplasmas (39%) confirmed by culture (Table 4). This observation was so consistent that we were able to predict, on the basis of observing rich populations of lactobacilli (not a mixed culture of Gram-positive and Gram-negative microorganisms) and unusual numbers of granulocytes on Gram-stains, which patients would show *Ureaplasma* on culture. There was, in fact, a 100% correlation with this observation. The *Ureaplasma*, which prefer a low pH, was in its best environment when there was a good population of *Lactobacillus* in the vagina. Data were also collected on age of the patient, whether the individual used birth control and what type, the number of pregnancies and live births, etc. These data are not presented because there was no correlation with these parameters and the main point of this presentation: That is, that all patients presenting with vaginal discharge in the presence of normal levels of lactobacilli and no *Candida*, were positive for ureaplasmas. Other patients with vaginal discharge for the most part presented with a mixed flora, most often containing members of the Enterobacteriaceae, a finding which so many others have observed. In the study in New York, looking at the incidence of ureaplasmas, a high proportion of infected patients complained of symptoms consistent with endometriosis. We wondered, therefore, whether there could be a causal relationship; such a relationship between *Ureaplasma* infection and endometriosis had not been proven by us or anyone else to date. One of the problems is that endometriosis is very much a clinical diagnosis with confirmation only by the post-mortem or post-hysterectomy pathological observation of the endometrium invading into the myometrium. The palpation of chocolate bodies in the cul de sac is considered diagnostic but occurs only in a small percent of patients. The problem is defining endometriosis in patients which do not present with such clinical findings which has made study difficult. A broad definition of endometriosis usually includes mostly young women, never before pregnant, who complain of chronic dysmenorrhoea. One woman presenting with years of dysmenorrhoea and a chronic infection with *Ureaplasma*, in the absence of other recognised vaginal

Table 4: PID and Flora

- *Candida* observed with lactobacilli in 60% of cases.
- GC and *Trichomonas* observed with mixed flora.
- Normal *Lactobacillus* populations, but vaginal discharge, and none of above, were positive for *Ureaplasma* (39% of remainder).

Figure 1: The reproductive tract from a breeding LEWIS female rat showing cystic ovaries as a result of bilateral salpingitis caused by *Mycoplasma pulmonis* infection.

pathogens, and was placed on hormonal therapy, was followed through her first pregnancy. A deep tissue specimen of the placenta, not contaminated by the vaginal flora, was obtained immediately following delivery and *Ureaplasma urealyticum* isolated. The baby was of normal weight, etc. and the gestation period was normal, or slightly extended. This observation led us to consider the role of the mycoplasmas/ureaplasmas in endometriosis and perhaps infertility which may have an animal equivalent, as described below.

MURINE MYCOPLASMOSIS

In a large colony of SPF Lewis rats (LEW), a disruption of the normal breeding colony productivity was seen over many months. This breeding problem was noted in the presence of chronic respiratory disease which was confirmed to be caused by *Mycoplasma pulmonis* (*Cassel* et al., 1976). Initially, we viewed the breeding problems as due to poor health in the rat colony as a result of the respiratory disease and attempts were made to treat this disease. However, necropsies of older breeding females disclosed the presence of ovarian cysts (Figure 1), the fluid from which contained pure cultures of *M. pulmonis*, and unilateral or bilateral salpingitis. The cysts and the associated salpingitis was clearly the cause of the drop in fertility, or complete infertility in

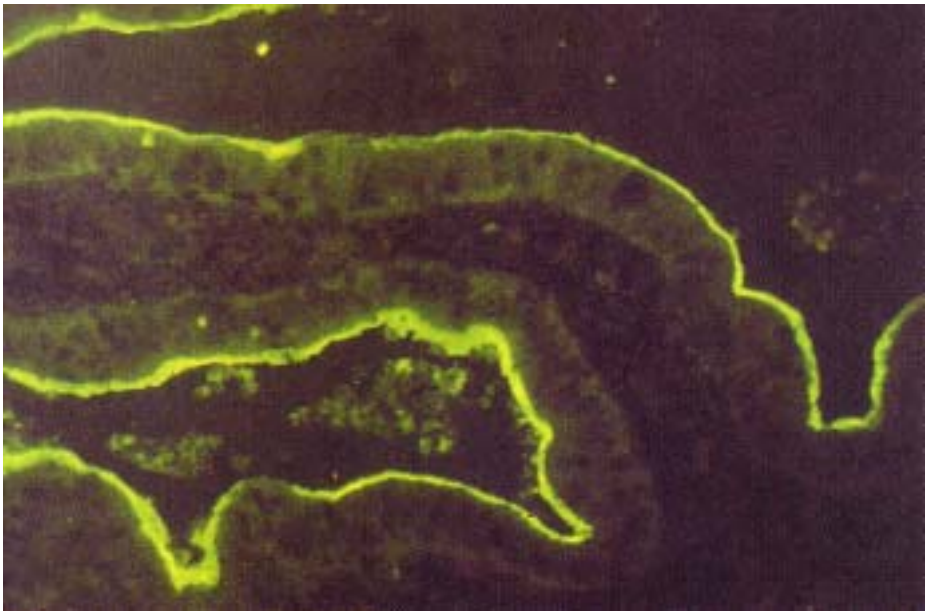

Figure 2: Colonisation of the uterine epithelium of Lewis rats by pure *M. pulmonis* (determined by fluorescence antibody).

the case of bilateral salpingitis. Histological examination showed colonisation of the uterine epithelium which proved to be pure *M. pulmonis*, determined by fluorescence antibody (Figure 2). The chronic infection of the reproductive tract led to metaplasia of the epithelium and even erosion. Curing the colony of *M. pulmonis* through caesarean section into a gnotobiotic environment resulted in a complete resolution of the breeding problem. While this is an animal model that is not the best reflection of what we currently know of the human situation, it does provide a pathogenic mechanism for the possible aetiology of endometriosis due to mycoplasmas or ureaplasmas. These organisms form very close associations with epithelial cell membranes, even penetrating them, and results in metaplastic changes which may permit such cells in the endometrium to invade into the myometrium causing the clinical presentation we call endometriosis.

DISCUSSION

There is controversy over whether ureaplasmas are frank pathogens able to cause clinical disease in women. Although it is quite well accepted that *Ureaplasma urealyticum* can cause nongonococcal urethritis (NGU) in men as a pure culture inoculated into volunteers, even its role in normally acquired NGU is being questioned. Data presented here argue for a role in PID since the organisms were consistently found in the presence of low vaginal pH, a rich population of lactobacilli and no other bacterial pathogens. Although it was not possible to exclude infection by *Chlamydia*, there are no reports of chlamydial infections of such high incidence which would totally account for

our observations. Methods to assess *Mycoplasma genitalium* infection were not available at the time of this work and infection with this organism cannot be excluded. Recent studies by others with this organism would suggest a direct role in PID and would be similar to the disease reported here in rats associated with a species of Mycoplasma. But again, the known incidence of *M. genitalium* in western populations would argue against it being the sole cause of the PID in our sample group.

CONCLUSIONS

This paper describes the results of a survey of over 380 women of childbearing age who were assessed for the cause of their respective pelvic inflammatory complaints or women who presented without health complaints. The data show a strong correlation between infection with *Ureaplasma urealyticum* and chronic vaginal discharge in the presence of an apparently normal population of vaginal lactobacilli. A suggestion of association with complaints consistent with endometriosis with *U. urealyticum* infection is noted. A rat model for the study of reproductive tract disease in humans caused by *Mycoplasma* is presented. The pathology observed in the LEW rat bears many similarities to human PID and may provide a controlled system for the assessment of pathogen virulence factors, pathogenesis and immunogenesis in PID.

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LITERATURE

Cassell, G.H., Carter, P.B., and Silvers, S.: *Mycoplasma pulmonis* genital tract disease in rats - development of an experimental model. Proc. Soc. Gen. Microbiol. 3, 150 (1976).